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**Official Organ of The American Phytopathological Society**

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# POTATO DISEASES AND HYBRIDIZATION

T. H. THUNG

(Accepted for publication January 25, 1947)

## INTRODUCTION

Economically the most important diseases of the potato crop in Java are the *Phytophthora* and wilt diseases. The latter, caused by *Bacterium solanacearum*, was known from different parts of Java long before the first was known. Reports about severe wilt were made by van der Goot (8) in 1924 and by Buijze (2) in 1929. The Phytophthora disease or blight has been reported from Java only since 1936 (5, 9); from that year onward losses due to this disease have been great. The statistical data indicate an important reduction in potato production; the production of Java in 1935 was 62,700 tons; in 1936, 1937, 1938, 1939, and 1940 it amounted, respectively, to 59,200 tons, 46,700 tons, 38,800 tons, 42,700 tons, and 40,500 tons (20). This reduction can only be explained as being due to losses caused by Phytophthora attacks. Many of the formerly well-known local varieties such as Preanger muis or Tenger muis, kentang wernie, hoei bien, and Franschen have disappeared because of their great susceptibility. More resistant varieties such as Eigenheimer and Bevelander are planted; and spraying with Bordeaux mixture is necessary to reduce the losses from *Phytophthora infestans*. Spraying makes the growing of the crop more costly but gives no absolute guarantee for a good yield, since all the known potato varieties are susceptible to wilt as well as to *Phytophthora*.

The wilt disease or brownrot causes not only damage to the stem but also a rot of the tuber; during storage of the potatoes great losses from tuber rot are common. The causal organism living in the soil remains virulent under favorable circumstances for several years. The possibilities of growing potatoes in noninfested soils are few, unless crop rotation is practiced and there are long periods without susceptible plants. Since many plants belonging to different families are host plants of *Bacterium solanacearum* (4) it is rather difficult to avoid reinfestation by the bacterium, susceptible plants in adjacent fields being a source of infection. Common crops such as tobacco, peanut, tomato, *Ricinus*, and beans are mentioned as host plants. Soil disinfection with sulfur, as practiced by Eddins (4) in Florida, gave no results in the Java soils (6), because of the difficulty of bringing these soils to such an acidity that *B. solanacearum* would be eliminated; even quantities as large as 1000 kilo sulfur powder per hectar (6) and 1 kilo per sq. m. (9) were insufficient. Wet rice cultivation has been observed to favor the elimination of soil infestation. Two reasons can be given: Firstly, an amelioration of soil structure can be effected by irrigation of fields during rice culture especially with water coming down from young volcanic areas and containing much silt. After the rice harvest the soil has to be tilled well and

dried. This amelioration, resulting in finer crumb texture and better aeration of the soil, favors the crop following the rice and does not favor the bacterium. Secondly, the antagonistic action between different soil microorganisms will be activated by irrigation. Thung (24) observed that *Protozoa* and many other organisms of wet fields reduce the numbers of *Phytophthora* and also of *Bacterium solanacearum*. Unfortunately the fields used for potato culture in Java are generally not to be irrigated because of lack of water nearby.

Desiccation of soil, too, may be an important control method. Nakata (11), studying tobacco wilt, found that a low water content was fatal to *Bacterium solanacearum* in several kinds of soil. By keeping the moisture of soil under this amount the bacterium may die out; this moisture level is low in sandy soil and high in clay soil. He reported that "the organism stops growth and gradually dies out when water content of soil reaches detrimental water content" of the organism, which corresponds with the "wilting coefficient" of Briggs and Schantz and "critical content residue" of Livingstone and Kokatsu for higher plants. Further he found that *B. solanacearum* grew only in soils with a pH between 6.1 and 8.1 and that "the organism is inhibited for its growth and finally dies out when cultured in the presence of *Aspergillus Oryzae*, *Actinomyces rutgerscens*, *Act. Californicus*, *Act. violaceus-ruber*, *Bac. mycoides*, *Bac. fluorescens*, *Bac. cereus*, *Bac. proteus*, and *Azotobacter chroococcum*." He advised: "(1) soil should be dried to reduce its water content to the detrimental water content of the organism, (2) the reaction of the soil should be adjusted not to keep pH 6.1 to 8.1 or (3) the soil should be inoculated with soil organisms inhibitory to the organism."

For the wet mountain climates of Java it is rather difficult to maintain this detrimental water content to such a depth that the soil will be freed of *Bacterium solanacearum*. In Java *B. solanacearum* tolerates a greater acidity *viz.* pH 4.5 (6); and sulfur in large quantities did not lower the acidity to such a degree as to eliminate *B. solanacearum*. The alkalinity of the soil can be heightened by lime supplements, but in practice this has not satisfactorily reduced wilt disease in the potato crops.

The antagonistic action of different microorganisms is to be stimulated by organic manures. Van der Poel (13) found a diminution of wilt disease in tobacco by supplying "katjang boengkil" (cake of ground peanuts after oil extraction) and ground parts of *Mimosa invisa*. But experiments with the same methods with potato in Java did not give satisfactory results (6).

The situation at present is that potato growing in Java is rather a risky affair. The production is not more than about 45 to 50 q. per hectar (20); in the temperate zones this production can be 4 to 6 times more (1, 12), partly because the potato grows better in the longer days of the temperate zone and partly because disease losses are less.

The possibility of finding resistant potato varieties has been studied and considerable work has been done already as regards resistance to *Phytoph-*

*thora*. A brief account has been given by Roemer, Fuchs, and Isenbeck (17).

## HYBRIDIZATION

In breeding for resistance against *Phytophthora*, *Solanum tuberosum* has been crossed with wild immune varieties. Müller (10) found the so-called W-races of potato, which proved to be resistant against different physiological races of *Phytophthora infestans*. But, unfortunately, since 1932 a new race (Phytophthora race S) causes great damage to these W-races.

Crosses between *Solanum demissum* (a resistant wild variety with 72 chromosomes) and *Solanum tuberosum* are being studied by several institutes. Reddick (15) and Schmidt (see 3) use successive backcrosses with *S. tuberosum*, and Salaman (18) uses selfed progenies of his crosses *S. demissum* × *S. tuberosum*.

A loss of resistance of *Solanum demissum* was observed with successive backcrosses to *S. tuberosum* (3). Crosses with other wild species such as *S. andigenum*, *S. semidemissum*, and *S. antipoviczii* (3, 7, 19, and 21) have been made.

Although many promising results have been secured, until now no commercial variety has been obtained from interspecific hybridization. Increased resistance found in some varieties of *Solanum tuberosum* has been obtained by Stevenson *et al.* (23) and by Reddick (14, 16) in crosses between varieties of *Solanum tuberosum*. Chippewa has been crossed with Katahdin (both commercial varieties), and Ekishirodzu, a Japanese resistant variety, with Evergreen. Promising seedlings came from a cross between the varieties No Blight and Katahdin (22). Stevenson *et al.* (23) mentioned also selection against *Phytophthora* by selfing a susceptible variety, by crossing a resistant variety and a susceptible one, and by crossing susceptible varieties.

Thus the resistance is brought to a higher degree, but immune varieties have not yet been obtained, nor varieties with such a resistance as is necessary to solve the blight problem, although many breeding products "are promising also from the standpoint of other characters of commercial importance" (22).

## PROBLEMS IN JAVA

In the temperate regions resistance is sought to Phytophthora attacks of the leaves, stems, and tubers. Tuber dry rot caused by *Phytophthora* is rather common in these potato-growing centers. This is not the case in Java; here very seldom tubers are found invaded by this fungus. It is also not known whether the same races of *Phytophthora infestans* prevailing in other countries occur in Java. The fact that certain potato varieties have a different reaction to the blight fungus in Java and in Europe shows that the aggressiveness in Java is different from that in Europe. The occurrence in Java of a new biologically specialized race or races since 1936 is not impossible, because Reddick (16) believed that *Phytophthora infestans* is

"moderately plastic." In Europe the potato variety Bevelander is more resistant to blight than Eigenheimer; but in Java the reverse is true. Such is also the case with Alpha and Robijn varieties which in Java are more susceptible than in Europe. In Java resistance to wilt disease is even more desirable than resistance to *Phytophthora* since no practical control methods for wilt are known. This resistance to wilt does not correlate with the resistance to *Phytophthora*. Varieties with some resistance, or perhaps less susceptibility, to *Bacterium solanacearum* are Arran Commander, Arran Consul, Botergele, Catriona, Gladstone, Golden Wonder, Green Mountain, Jubel, Kentang Djawa, Koninkjes, Paul Krüger, Roode Star, Sutton Flourball, Woeloeng, and M. 19; they are more resistant than Bevelander. Kentang Djawa and Woeloeng are local varieties found only in remote districts, M. 19 is a newly imported variety not yet identified (when imported the name was not known). Also, Eddins (4) found Green Mountain to be one of the more resistant varieties. Many of the varieties mentioned have been found in Java to be more susceptible to *Phytophthora* than Bevelander (see table 1). The following potato varieties received from Eddins in California have been observed in California as well as in Java to be fairly resistant to blight, but they are susceptible to *B. solanacearum* in Java: 0–55 (Chippewa × Katahdin), 1173–32, 1173–72, 1173–83, 1173–92, and 1173–136 (Spaulding Rose × Katahdin).

TABLE 1.—*The reactions of potato varieties to infection by Phytophthora infestans in relation to the reaction of the variety Bevelander*

| Variety | Disease reaction[a] | Variety | Disease reaction[a] |
|---|---|---|---|
| Ally | +0.4 | Sutton Flourball | −0.2 |
| Abundance | +1.2 | Sutton Ninetyfold | +0.2 |
| Americana | +2.2 | Sutton White City | +0.2 |
| Alpha | +0.9 | Thorbecke | −0.1 |
| Australia 981 | +0.6 | Triumph | +0.3 |
| Australia 982 | +0.4 | Up to date | +3.0 |
| | | Zonda di Berlino | +1.8 |
| Arran Commander | −1.0 | | |
| Arran Comrade | +1.4 | 172—18[b] | −0.4 |
| Arran Pilot | +1.5 | 174—29 | −0.5 |
| Arran Crest | +0.4 | 175—37 | −1.1 |
| Arran Cairn | +0.3 | 175—47 | −1.1 |
| Arran Chief | −0.3 | 175—52 | −0.9 |
| Arran Consul | −0.5 | 175—55 | −1.2 |
| Arran Peak | +1.4 | 1109—8596 | −0.2 |
| Arran Banner | +1.7 | | |
| | | M. 8[c] | −0.3 |
| Bintje | +2.2 | M. 9 | 0.0 |
| Bine fantina di bhiaggia | +2.2 | M. 10 | +0.3 |
| Basilicata | +2.2 | M. 13 | 0.0 |
| British Queen | +2.2 | M. 14 | +0.4 |
| Duke of Kent | −0.8 | M. 18 | +0.1 |
| Duke of York | +1.4 | M. 19 | −0.1 |
| Di Vernon | +2.2 | M. 24 | +0.4 |
| Donkere Roode Star | +0.8 | M. 27 | +0.1 |
| Doon Star | −0.5 | M. 28 | −0.2 |
| Donnontar Castle | +0.9 | M. 30 | +0.2 |
| Eigenheimer | −0.2 | M. 32 | +0.1 |
| | | M. 33 | +0.1 |

TABLE 1—(*Continued*)

| Variety | Disease reaction[a] | Variety | Disease reaction[a] |
|---|---|---|---|
| Epiquire | +0.9 | M. 38 | −0.1 |
| Eclipse | +1.6 | M. 47 | −0.2 |
| Express | +1.6 | M. 47/77 | −0.2 |
| Ella | +1.9 | M. 50 | −0.3 |
| Energie | +1.1 | M. 56 | −0.1 |
| Frühmölle | +0.7 | M. 58 | −0.5 |
| Franschen | +2.9 | M. 60 | −0.2 |
| | | M. 67 | −0.1 |
| Furore | −0.4 | M. 71 | −0.4 |
| Glasgow Favorite | +0.2 | M. 73 | −0.3 |
| Gladstone | −0.2 | | |
| Golden Wonder | +0.4 | M. 77 | −0.2 |
| Geeltjes | +3.5 | M. 139 | +1.5 |
| Hellena | −0.5 | M. 164 | 0.0 |
| Industrie | −0.8 | M. 165 | +0.4 |
| | | M. 167 | +0.4 |
| Inv. Favorite | +0.1 | M. 168 | −0.6 |
| Jubel | +0.1 | M. 170 | −0.2 |
| King Edward | +0.8 | M. 173 | −0.1 |
| King George | +0.2 | M. 175 | +0.3 |
| Limosa | +3.4 | M. 177/147 | −0.4 |
| Mathilde | −0.3 | M. 179 | +0.5 |
| Majestic | +1.5 | M. 180 | +0.3 |
| N. Zeeland | −0.3 | M. 181 | +0.6 |
| Noordeling | −0.6 | M. 182 | +0.1 |
| Nationaal | −0.2 | M. 182b | +1.1 |
| Populair | −0.5 | M. 183 | +0.5 |
| Pisana | +0.5 | M. 184 | +0.6 |
| Parnassia | −0.6 | M. 190 | −0.2 |
| Robijn | +0.2 | M. 192 | 0.0 |
| Red King | +2.2 | | |
| Red Skin | +0.2 | 1173—32[d] | −1.3 |
| Record | −0.2 | 1173—72 | −0.3 |
| | | 1173—83 | −0.1 |
| S. Castle | +1.2 | 1173—92 | −0.3 |
| S. Coprina | 0.0 | 1173—136 | −0.3 |
| S. Bellahouston | +0.2 | 055 | −0.2 |

[a] A plus rating indicates that the variety was more susceptible than Bevelander; a minus rating that it was more resistant.

[b] Hybrids from Russia, progeny of backcrosses *Solanum demissum* × *S. tuberosum*.

[c] M numbers are newly imported, not yet identified varieties.

[d] Crosses of *Solanum tuberosum* × *S. tuberosum* received from California.

Therefore these two diseases present different problems; it is desirable to have potatoes immune from both, but this would complicate the matter much more. The main problem for the temperate zones is the immunity from *Phytophthora;* in Java we must seek first the immunity from wilt disease.

## METHODS OF TESTING

The testing for resistance against *Phytophthora infestans* is done in a field where the conditions for blight epidemics are very favorable during a large part of the year, the air humidity being high.

The varieties to be tested are planted in rows separated by rows of the

variety Bevelander. The severity of attack is indicated by numbers ranging from 0 to 6. The inspection for disease rating is made 20, 30, 45, and 60 days after planting; the second and third ratings, being the most important, are valued twice as much as the others (6). The average of these ratings is compared with that of the variety Bevelander; in this way several varieties of *Solanum tuberosum* and of *S. demissum* hybrids received from Russia have been listed (Table 1). Plants were not sprayed with Bordeaux mixture.

Resistance to *Bacterium solanacearum* is tested at another place on severely infested soil, where 20 tubers of each variety are planted in rows. The tubers of the different varieties to be tested and those of the standard variety Bevelander are alternated at random. The main test for the market varieties is the yield of tubers as it is related to tuber rot during storage. The test for the wild varieties and for the hybrids has for its base the longevity of the plants. When more than 50 per cent of the planted variety remains healthy during at least 2 months, this variety is to be regarded as resistant, because during this time from 80 to 100 per cent of the variety Bevelander generally has become diseased. This test is repeated at least twice owing to the variability of the occurrence of wilt in the experimental plants. During the rainy seasons many more diseased plants generally are to be found than during the dry monsoon. The average number during a whole year indicates the grade of resistance. The standard varieties of *Solanum tuberosum* usually had more than 50 per cent wilt, and often 80–100 per cent wilt; and they are to be regarded as susceptible. Spraying with Bordeaux mixture is done regularly.

## RESULTS

Besides the resistance of potato varieties to *Phytophthora* and wilt diseases, the resistance of different wild species of *Solanum* has been studied. Through the kindness of several foreign institutes the Institute for Plant-diseases now has available several wild species. Most of them are blossoming well under tropical conditions. The reverse is true for most of the varieties of *S. tuberosum*. Thus it has been necessary that part of the hybridization work be done in a temperate climate, in the Netherlands; this was the case with most of the crosses between wild species and *S. tuberosum*. The backcrosses with *S. tuberosum* as well as the reciprocal crosses have been done in Java. Varieties that blossom rather well in Java are: Americana, Arran Commander, Arran Cairn, Bevelander, Djawa, Duke of Kent, Eigenheimer, Industrie, Jubel, Pizona, Populair, Robijn, Sutton Flourball, Thorbecke, and Triumph.

The progenies of crosses with the following species of *Solanum* have been tested for their disease resistance: *S. andigenum*, *S. antipoviczii*, *S. caldasii*, *S. chacoense*, and *S. demissum*.

It is known that some of these potato species comprise several races. Schick and Schaper (see 19) showed the differences among several races of

TABLE 2.—*The resistance to Phytophthora infestans in 4 wild species of Solanum and in $F_1$ hybrids obtained when the species were crossed with S. tuberosum*

| Parental wild species of *Solanum* | $F_1$ hybrids[a] with *Solanum tuberosum* | Resistance to *Phytophthora* in field tests | |
|---|---|---|---|
| | | Wild species | Hybrids |
| | *Number* | *Per cent* | *Per cent* |
| *S. andigenum* | 82 | 80 | 29 |
| *S. antipoviczii* | 6 | 100 | 33 |
| *S. caldasii* | 33 | 90 | 39 |
| *S. demissum* | 500 | 100 | 33 |

[a] These hybrids are $F_1$ progenies of hybridization with the wild species as female parent.

*Solanum demissum* in their resistance to four races of *Phytophthora infestans*. *S. andigenum* is also very heterogeneous (7, 19).

The wild species used in the hybridization work of the Institute for Plant-diseases proved, according to our testing experiments, very resistant to *Phytophthora* disease and also to wilt. An exception to this is *Solanum demissum;* the several races of the species available at the Institute were immune from *Phytophthora* but were not resistant to *Bacterium solanacearum*. The numbers for their percentages of resistance (Tables 2 and 3) were fixed after yearly experiments during five years.

Bukasov (3), mentioning the data concerning hybridization experiences of different institutes, reported the results of E. Schmidt, who found that in the $F_1$ hybrids between *Solanum demissum* and *S. tuberosum* there was 100 per cent resistance, even to the especially virulent Streckenthin race of *Phytophthora infestans*, but that the resistance was less in the later hybrids secured from backcrosses to *S. tuberosum*. Reddick (15) recorded a case in which 50 per cent of the double backcross plants of *S. demissum* proved to be immune from blight under North American conditions. Our own data for the $F_1$ hybrids of the wild *Solanum*-species × *S. tuberosum* are in table 2.

Table 3 gives the data on resistance to *Bacterium solanacearum* in the same $F_1$ progenies and in progenies of further backcrosses with *Solanum tuberosum* and of reciprocal crosses.

TABLE 3.—*The resistance to Bacterium solanacearum in 5 wild species of Solanum and in hybrids obtained from crosses between the wild species and S. tuberosum*

| Wild species of *Solanum* | Resistance of the wild species | Resistance of the hybrids from crosses | | |
|---|---|---|---|---|
| | | Wild species × *S. tuberosum* | (Wild species × *S. tuberosum*) × *S. tuberosum* | *S. tuberosum* × (wild species × *S. tuberosum*) |
| | *Per cent* | *Per cent*[a] | *Per cent*[a] | *Per cent*[a] |
| *S. andigenum* | 80 | 31 (153) | 58 (24) | |
| *S. antipoviczii* | 80 | 24 (25) | 57 (7) | |
| *S. caldasii* | 70 | 33 (55) | 54 (222) | 53 (101) |
| *S. chacoense* | 70 | 36 (25) | 50 (28) | 43 (77) |
| *S. demissum* | 40 | 18 (1369) | 53 (65) | 54 (101) |

[a] The number in parentheses is the number of hybrid varieties tested.

The testing for resistance to *Phytophthora infestans* has not yet proceeded so far as that for wilt disease owing to the fact that every hybrid variety yields only a limited number of tubers, which first are used in the experiments concerning wilt resistance.

The $F_1$ progeny have a diminished resistance when compared with the original wild parent. According to the experience of other institutes this is to be expected regarding the resistance to *Phytophthora*, but it was not known for *Bacterium solanacearum*. Promising are the backcrosses with *Solanum tuberosum* and also the reciprocal crosses since they do not show further diminution of resistance. There is even an increase of resistance when these progenies are compared with the $F_1$ progenies. This can probably be ascribed to genetic factors, since the external conditions for wilt disease were favorable. This will be tested further during the next several years. Several of the resistant progenies, especially those of the crosses *S. tuberosum* × (wild species × *S. tuberosum*), yield well-formed tubers with rather good taste and flat eyes. The skin colors are different, varying from white to rose and dark purple, the flesh varying from white to yellow.

Reddick (15) came to the conclusion with his *Solanum demissum* crosses: "If an immune first-generation plant is backcrossed with pollen from a cultivated variety the progeny is heterogeneous." We obtained the same results with regard to the resistance to wilt with all the crosses. One backcross can yield highly resistant and immune hybrids, the next backcross susceptible ones.

From the data given, our work program has proved to be safe: Still starting from resistant hybrids we shall make further backcrosses with cultivated varieties until plants will be obtained with tubers of commercial size and with high resistance, in the first place, to *Bacterium solanacearum*. Many of the successive progenies will have mostly wild features, as we found in former crosses, but some will approximate the desired characters. It is of great importance to work with large numbers.

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# LEAF BLIGHT OF PINK CALLA CAUSED BY PHYTOPHTHORA ERYTHROSEPTICA

C. M. TOMPKINS AND C. M. TUCKER[1]

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## INTRODUCTION

A serious leaf blight of the Superba variety of pink calla (*Zantedeschia rehmannii* Engler) was first observed during the summer of 1938 in field plantings in the Monterey Bay region of California. The disease has also been prevalent in subsequent years, causing appreciable loss wherever found. The symptoms of this disease, the causal organism, host range, and suggestions for control are discussed in this paper.

## REVIEW OF LITERATURE

In 1912, Pethybridge (5) described a new tuber rot of the potato (*Solanum tuberosum* L.) which was first observed in 1909 at Clifden, County Galway, Ireland. A more detailed account (6) followed in 1913, in which he suggested the common name of pink rot, descriptive of invaded tissues which, on exposure to the air, turned pink but later became dark-brown or black. In the same paper, he proposed that the causal fungus be named *Phytophthora erythroseptica* Pethyb. Still later, Pethybridge (7) mentioned wilting of the foliage and stalks above ground, rotting of the stalks below the ground level, and partial decay of some of the roots and rhizomes, in addition to tuber rot, as conspicuous phases of the disease. Therefore, he suggested that the common name "pink-rot" be changed to "pink-rot-wilt." Two decades later, Cairns and Muskett (3) corroborated the facts as presented by Pethybridge (5, 6, and 7), emphasizing that normally the disease is of importance only as a tuber disease. High humidity and poor aeration were also shown to cause heavy loss in affected crops in storage. According to Cairns and Muskett (3) and Tucker (10), the disease has been reported from Scotland, England, Wales, Holland, Bulgaria, and Java, Netherlands East Indies. In 1944 *P. erythroseptica* was isolated from tubers in Idaho by E. C. Blodgett, and in 1945 isolates of the species from tubers in Nebraska were received from R. W. Goss. In both instances the species was identified by one of the writers. Recently, White (13) reported the disease in Tasmania.

Westerdijk and van Luijk (12) reported a root rot of *Atropa belladonna* L., caused by *Phytophthora erythroseptica*, in Holland in 1920. Later, in 1926, Alcock (1) observed that this fungus attacked the base of the stem of this host in early summer in England. Above the point of attack, severe wilting of the foliage occurred. The fungus also caused damping-off of

[1] Joint contribution from the Division of Plant Pathology, California Agricultural Experiment Station, and the Department of Botany, Missouri Agricultural Experiment Station.

belladonna seedlings. Alcock suggested the name *P. erythroseptica* var. *atropae*, but Tucker (9) considers the varietal name unwarranted.

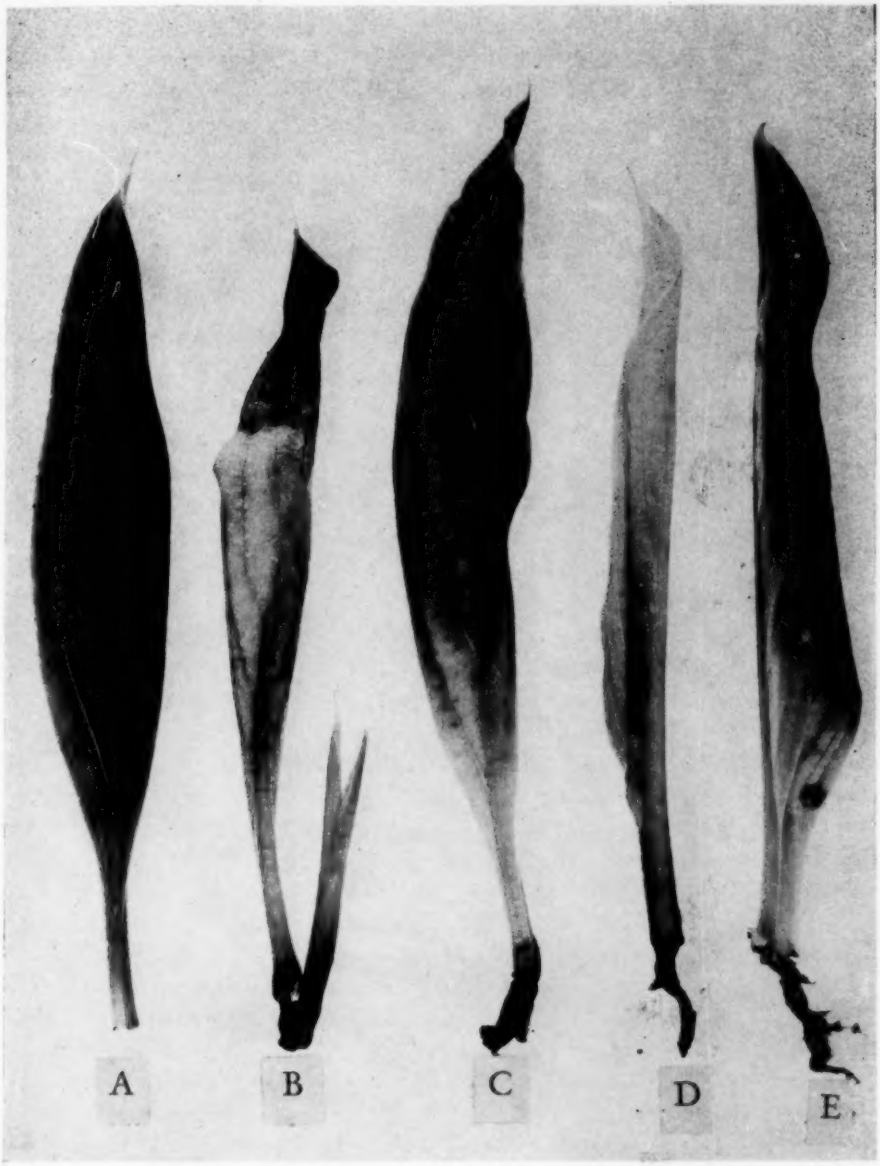


FIG. 1. Leaves of pink calla, variety Superba, grown from flowering-size corms. A. Healthy leaf from control plant. B, C, D, E. Four leaves having a brownish-black discoloration of the lower part of the petioles, with chlorosis and distortion of the leaf laminae, 22 days after inoculation of the plant with *Phytophthora erythroseptica*.

In 1938, Buddin (2) described a root rot, shoot rot, and shanking of tulip (*Tulipa gesneriana* L.), caused by *Phytophthora cryptogea* Pethybr. and

Laff. and *P. erythroseptica*, which had caused great damage in greenhouse-forced crops.

### SYMPTOMS OF THE DISEASE

With the onset of the disease, the clusters of narrow, green leaves of pink calla plants, grown either from seeds or corms, suddenly show a general

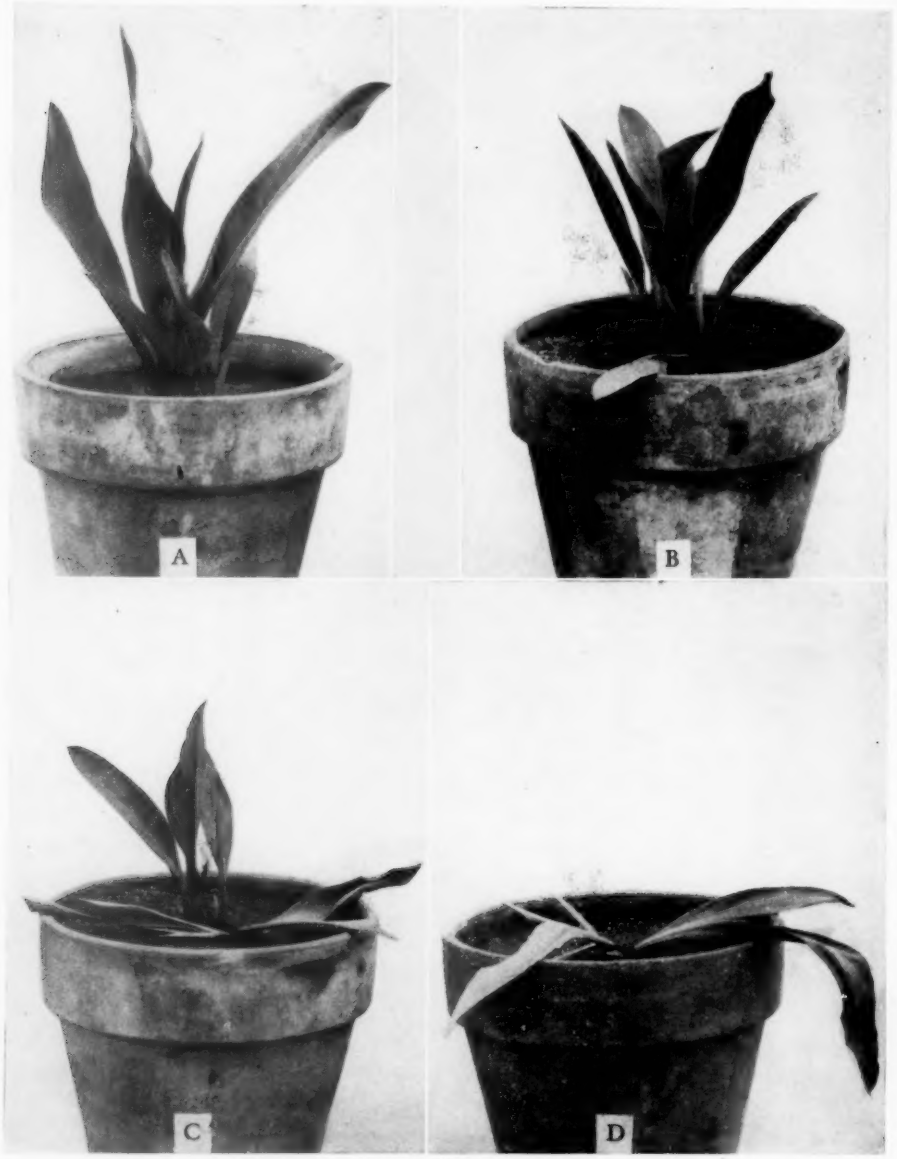


FIG. 2. Pink calla plants, variety Superba, grown from flowering-size corms. A. Healthy control plant. B, C, D. Diseased plants, showing progressive symptoms, from mild to severe, 22 days after inoculation with *Phytophthora erythroseptica*.

chlorosis and are less turgid than healthy specimens. Infected leaves soon turn yellow and become distorted, with a tendency for the edges to curl upward. Simultaneously, for a distance of several centimeters above and below the surface of the soil, a wet, odorless, brownish-black discoloration of the petioles is noticeable (Fig. 1). At first, this is firm but soon turns soft and becomes mushy. With the rapid destruction of the supporting tissues in the petioles, the leaves collapse, singly or in groups, until all in the cluster are prostrate. This occurs within a few hours, and death of the foliage ensues within a day or two (Fig. 2). Even in the most severe cases, no infection of the roots or corms of blighted plants has been observed.

Apparently the disease is favored by cool, foggy weather, excessive irrigation, and heavy, poorly drained soils.

### THE CAUSAL FUNGUS, PHYTOPHTHORA ERYTHROSEPTICA

Isolations made from diseased pink calla leaves on malt-extract agar have consistently yielded a fungus which has been identified as *Phytophthora erythroseptica* Pethybr.

Cultures on the usual media incubated at room temperatures seldom develop fruiting bodies with the exception of occasional hyaline, spheroidal structures regarded as poorly differentiated chlamydospores. Cultures on oatmeal agar incubated one month at 8° C. develop sporangia sparingly, and oogonia, oospores, and antheridia very abundantly. Morphologic studies were made on 2 isolates which proved indistinguishable.

The sporangia are hyaline, ellipsoidal to obpyriform, terminal on long sporangiophores not differentiated from the vegetative hyphae or on short lateral branches, nonpapillate, but often provided with a lenticular hyaline plug at the slightly flattened apex; 29–39 × 15–26 $\mu$, mean 34.6 × 20.7 $\mu$. Germination occurs by germ tubes or zoospore formation; in the latter case, evacuation of the sporangium is frequently followed by renewed growth of the sporangiophore through the base of the sporangium, and the development of another sporangium within or beyond the first.

Sexual reproduction occurs in great profusion. The oogonia are hyaline, spheroidal, tapering abruptly to a slender stalk completely encompassed by the persistent, amphigynous antheridium; 27–44 $\mu$ in diameter, mean 35.8 $\mu$. Oospores single, spheroidal, cream to straw-color, wall thick (3–4 $\mu$), contents densely granular, occurrence of reserve globules irregular and varying with age of spores; germination was not observed; 20–36 $\mu$ in diameter, mean 29.4 $\mu$. Antheridia roughly spheroidal, but frequently irregular and distorted, sometimes with one or two finger-like protuberances, amphigynous, intimately and permanently enclosing the slender oogonial stalk; 12–19 $\mu$ in diameter.

The oogonia and oospores correspond closely in size to the description by Pethybridge (6), but are slightly smaller than those reported by Tucker (9); their abundant development in the calla isolates may have been a factor in determining size. Comparison of the calla isolates with the Idaho and

Nebraska isolates from potato tubers, both of which produce oogonia in large numbers, failed to reveal significant differences in morphology. The similarity of the isolates in temperature-growth relations provides additional evidence of their relationship.

The calla isolates may be distinguished from the potato isolates of *Phytophthora erythroseptica* by their failure to invade and cause a pink rot of potato tubers. Tubers inoculated at incisions with the potato isolates were almost completely invaded after one week; those inoculated with the calla isolates did not develop symptoms of invasion. Tucker (9), using isolates of *P. cactorum* (L. and C.) Schroet., *P. palmivora* Butl., and *P. parasitica* Dast. from numerous hosts, showed that isolates of the 3 species from different origins may vary widely in pathogenicity. Alcock (1) reported that an isolate of *P. erythroseptica* from *Atropa belladonna* caused a rot of potato tubers which did not develop the pink color characteristic of infections by potato isolates. The failure of the calla isolate to infect potato tubers is evidence of the occurrence of physiologic specialization in *P. erythroseptica*, but does not provide a valid basis for its consideration as the type of a new species or variety.

Isolates of the fungus proved pathogenic to healthy pink calla plants grown from healthy corms. Inoculum was prepared by growing the fungus on sterilized cracked wheat in 8-inch test tubes. When ready for use, this was added to autoclaved soil contained in 6-inch pots in such a manner as to avoid injuring the root system of the young plants. Sterile cracked wheat was added to the pots serving as controls. The air temperature in the greenhouse in which the tests were conducted, ranged from 13° to 19° C. All pots were heavily watered each day in order to keep the soil very moist and thus provide optimum conditions for infection. The incubation period ranged from 22 to 29 days, but all leaves of infected plants died within a day or two after the foliage had collapsed (Figs. 1 and 2). Of 30 plants inoculated, none escaped infection, while the 30 control plants remained healthy. The fungus was reisolated from several petioles of each infected plant, and it proved to be identical with the original isolate. The reisolates proved highly pathogenic. In these tests, the infected plants exhibited symptoms identical with those of naturally infected plants. In all infection tests, the corms which had produced blighted leaves remained firm and healthy.

The relation of temperature to growth of the mycelium was studied. The culture tubes (2.1 by 20 cm.) used, and the procedure followed, were those previously described by Tompkins and Gardner (8). The medium used was malt-extract agar, pH 7.0. Inoculated tubes were kept at room temperature for 48 hours. Then 3 tubes of the isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 4° to 40° C. The cultures were incubated for 96 hours. The cardinal temperatures were determined on the extent of mycelial growth in the culture tubes.

The minimum temperature for growth of the pink-calla isolate of *Phy-*

*tophthora erythroseptica* was approximately 13° C., the optimum 25°, and the maximum 28°. Using a strain of the fungus isolated from potato in Holland, Tucker (9) observed that it grew only slightly on corn-meal agar at 15°, profusely at 27.5°, and failed to develop at 30°. Thus, the temperature relations of the pink-calla isolate are in close agreement with those of the potato isolate. In their studies, Cairns and Muskett (4) showed that the minimum, optimum, and maximum growth temperatures of a potato isolate were 5°, 25°, and 31° C.

## EXPERIMENTAL HOST RANGE

A survey of the literature indicates that the host range of *Phytophthora erythroseptica* is extremely limited. In laboratory tests, Pethybridge (6) obtained artificial infection of aseptically-prepared blocks of living tissue of mangel (*Beta vulgaris* L.), white turnip (*Brassica rapa* L.), and Swede (*B. campestris* L. var. *napo-brassica* DC.), but failed with carrot (*Daucus carota* L.) and parsnip (*Pastinaca sativa* L.). Swedes and white turnip roots were successfully infected through wound-inoculation; the fungus was highly pathogenic to turnip. Tucker (9) found the species virulently pathogenic to wounded green fruits of tomato (*Lycopersicum esculentum* Mill.), and weakly pathogenic to wounded apple fruits (*Pyrus malus* L.).

Limited studies on the host range of the pink-calla isolate of *Phytophthora erythroseptica* were conducted in the greenhouse and laboratory. In the former, using the method of inoculation described for the tests on pathogenicity, 6 yellow calla plants (*Zantedeschia elliottiana* Engler) of 8 inoculated were infected after 28 days. The foliage symptoms were comparable to those obtaining on infected pink-calla plants. No infection was obtained on plants of white calla (*Z. aethiopica* Spreng.),[2] Fiery Blood Red annual stock (*Matthiola incana* R. Br. var. *annua* Voss), tomato (*Lycopersicum esculentum* Mill.), or China aster (*Callistephus chinensis* Nees). In the laboratory, no infection was obtained by placing agar inoculum on the surface of healthy white calla rhizomes, on corms of pink and yellow calla, and tubers of tuberous-rooted Begonia (*Begonia tuberhybrida* Voss) and White Rose potato.

## DISCUSSION

Although infection of pink calla plants by *Phytophthora erythroseptica* is chiefly characterized by wilting and subsequent death of the foliage, apparently the fungus is unable to parasitize the roots and corms. However, additional loss is reflected in the condition of the mother corms which were used originally as planting stock. As a result of foliage failure, especially if it occurs early in the season, there is little or no increase in the size of the mother corms, and no new offsets or cormels are formed. After harvesting and curing, the mother corms from infected plants may again be used as planting stock, but undoubtedly their vigor has been reduced.

[2] After completion of this manuscript, *Phytophthora erythroseptica* was isolated at harvest time from diseased white calla rhizomes grown on the San Francisco Peninsula. A full report on this disease will be published in the near future.

Avoidance of this disease can best be obtained by careful selection of clean, well-drained soils and by the judicious use of irrigation water. These factors are of paramount importance in averting losses. Pethybridge (6, 7), Cairns and Muskett (3, 4), and Van Haeringhen (11) made similar recommendations relative to the control of pink rot of potato.

## SUMMARY

A leaf blight of pink calla is prevalent in the Monterey Bay region of California.

Symptoms of the disease consist of rapid wilting, yellowing, and collapse of the leaves, with blackening of the petioles at and slightly above and below the soil level. Death of the foliage occurs in a few days.

The causal organism has been identified as *Phytophthora erythroseptica* Pethybr. on the basis of morphologic characters and temperature-growth relations. It differs from isolates from potato in pathogenicity, failing to infect and cause a pink rot of potato tubers.

In the greenhouse, infection was obtained by adding the fungus to the wet, autoclaved soil of potted plants grown from healthy corms. The incubation period averaged 25½ days.

The pink-calla isolate of the fungus was pathogenic to yellow-calla potted plants.

The minimum temperature for mycelial growth was 13° C., the optimum 25°, and the maximum 28°.

The disease is favored by wet, poorly drained soil and by cool, foggy weather. Crop rotation and sanitation are suggested as a method of control.

This is apparently the first record of *Phytophthora erythroseptica* as a pathogen on pink calla.

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# ACCELERATION AND RETARDATION OF GERMINATION OF SOME VEGETABLE SEEDS RESULTING FROM TREATMENT WITH COPPER FUNGICIDES[1]

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In recent years the use of fungicides to protect vegetable seeds and seedlings from attack by microorganisms has become an economic necessity for the commercial production of many crops. The literature of plant pathology has reported enough information to indicate that the value of treatments to seed varies with experimental conditions. A chemical which works well on one kind of seed may be useless or dangerous on another. The fungicidal power of a compound against one organism is often no criterion of its strength against a different pathogen. Many other factors such as soil pH, temperature, water, age of seed, and light intensity complicate the picture further. The purpose of this study is to explain some differences observed in the effect of two fungicides containing copper on seven kinds of vegetable seeds.

## LITERATURE REVIEW

Previous workers have reported injury by fungicides containing copper to seeds of peas (3, 10, 11, 12, 13, 15, 24, 25, 26, 37, 41), cucumbers (32), and crucifers (7, 8, 23). There are other crops, however, on which consistently beneficial results of fungicides containing copper have been obtained. Beets (14, 19, 24, 25), spinach (8, 9, 16, 36), eggplant, pepper and tomato (33, 34) are examples. The authors who report these results make no claim of benefit to the seeds, but attribute all of the success to protection from attacks by fungi.

The evidence presented here shows that the effect of copper compounds on vegetable seeds may be either to accelerate or retard germination. The nature of the enzyme systems responsible for respiration in the seeds determines whether the action of copper is beneficial or injurious. One enzyme system present in barley (30, 31), oats (4), cucumbers (2), and peas (17, 21) requires the presence of sulfhydryl groups in one or more of its enzymes. Enzymes of this type are inhibited by copper (1, 20, 22, 29, 35, 39). A different enzyme system found in potatoes (38), beets (42), spinach (5), and mushrooms (27) contains the enzyme tyrosinase, which requires the presence of copper ions for its activity. Plants of this latter type might be benefited rather than injured by moderate amounts of copper.

## MATERIALS AND METHODS

The seven kinds of vegetable seeds[2] listed in table 1 were immersed in 1.5 per cent copper sulfate solution at room temperature for one hour. A

[1] Excerpt from a thesis presented December, 1945, to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Furnished by the Cooperative G.L.F. Mills, Ithaca, New York.

duplicate set was revolved in Erlenmeyer flasks for ten minutes in the presence of the quantities of cuprous oxide[3] listed in table 1.

Germination tests were made with lots of 60 seeds each that had been stored for varying times at controlled humidities and temperatures. Twenty from each lot were placed on moist filter paper in Petri plates in the laboratory, 20 were planted in the greenhouse in steamed soil, and 20 were planted in artificially infested greenhouse soil containing parasitic strains of *Pythium, Corticium,* and *Fusarium.*

Germination was counted daily for two weeks in the laboratory (three weeks for eggplant and pepper), and seedling emergence counted daily in the greenhouse for three weeks (four weeks for eggplant and pepper). A daily count of post-emergence damping-off was also made. The figures for emergence in infested soil represent final stands at the end of the germination period. The others are total emergence. Coefficients of velocity calculated by the Kotowski (28) method give a measure of speed of germination.

TABLE 1.—*Dosage of cuprous oxide used in treating seven kinds of vegetable seeds*

| Kind | Variety | Dosage, per cent by weight |
|---|---|---|
| Beet | Detroit Dark Red | 1.13 |
| Cabbage | Glory of Enkhuisen | 0.75 |
| Cucumber | Arlington White Spine | 0.38 |
| Eggplant | Black Beauty | 0.75 |
| Pea | Telephone | 0.38 |
| Pepper | California Wonder | 0.75 |
| Spinach | Bloomsdale | 0.75 |

Plantings were randomized and significance of differences determined by analysis of variance.

A second test was conducted in which ten, 50-seed lots of peas and ten, 100-seed lots of spinach were planted in steamed soil. A duplicate series was placed in Petri plates. Cuprous oxide was used at the same dosage as before and records taken in the same manner.

For studies of oxygen uptake, samples of seeds and seedlings ground in a mortar were diluted to 10 per cent by weight with a solution containing M/20 sodium succinate and M/30 phosphate buffer at pH 6.5. The suspensions were strained through a double layer of fine cheesecloth and placed in Fenn differential volumeters. Cuprous oxide at a concentration of 0.007 per cent was added to the ear of the vessel for studies of respiratory inhibition.

Imbibition by seeds or seedlings was determined by recording the difference between fresh weight and dry weight after heating to 80° C. in a vacuum oven for 24 hours.

Copper was determined by the Callan and Henderson (6) method. To a slightly ammoniacal tissue suspension 10 per cent by volume of a 0.1 per cent sodium diethyl-dithio-carbamate solution was added. Color intensity was measured in a Cenco-Shear-Sanford photelometer with a No. 1 blue filter.

[3] "Yellow cuprocide" furnished by Rohm and Haas Company, Philadelphia, Pennsylvania.

Sulfhdryl groups were determined by the nitroprusside method (18, 40). Two grams of ground tissue were taken up in 5 ml. distilled water. Five drops of 10 per cent sodium nitroprusside solution, 2 g. of solid ammonium sulfate, and 3 ml. of 10 per cent acetic acid were added and the tube was shaken. Addition of an excess (one ml.) of concentrated ammonium hydroxide caused a pink coloration in the presence of sulfhydryl groups.

## EXPERIMENTAL RESULTS

The results of germination tests of seeds treated with cuprous oxide and copper sulfate are presented in table 2. The seeds can be divided into two groups based on their reaction to the fungicide in the absence of pathogens. Beet, eggplant, pepper, and spinach germination is increased or hastened in one or more cases by treatment. Cabbage, cucumber, and pea germination,

TABLE 2.—*The germination of seven kinds of vegetable seeds dusted with cuprous oxide or soaked in 1.5 per cent copper sulfate solution. (Means of 24 samples, 20 seeds each)*

| Kind of seed | Treatment | Petri plates | | Emergence from soil | |
|---|---|---|---|---|---|
| | | Germination, per cent | Coeff. of velocity | Steamed | Infested |
| Beet | Copper sulfate | 59 | 20.0 | 133 | 74 |
| | Cuprous oxide | 50 | 19.8 | 117 | 77 |
| | None | 37 | 22.4 | 123 | 49 |
| | Least significant dif. at 19 : 1 | 10 | N.S. | N.S. | 16 |
| Eggplant | Copper sulfate | 56 | 16.0 | 60 | 36 |
| | Cuprous oxide | 59 | 16.1 | 64 | 41 |
| | None | 48 | 13.1 | 63 | 22 |
| | L.S.D. at 19:1 | N.S. | 2.3 | N.S. | 9 |
| Pepper | Copper sulfate | 62 | 11.7 | 62 | 52 |
| | Cuprous oxide | 70 | 12.1 | 64 | 35 |
| | None | 63 | 10.7 | 61 | 22 |
| | L.S.D. at 19 : 1 | N.S. | 1.2 | N.S. | 11 |
| Spinach | Copper sulfate | 50 | 17.2 | 65 | 32 |
| | Cuprous oxide | 38 | 16.6 | 67 | 28 |
| | None | 22 | 20.6 | 64 | 20 |
| | L.S.D. at 19 : 1 | 13 | N.S. | N.S. | 9 |
| Cabbage | Copper sulfate | 8 | 47.0 | 29 | 42 |
| | Cuprous oxide | 65 | 35.4 | 60 | 42 |
| | None | 68 | 40.1 | 61 | 56 |
| | L.S.D. at 19 : 1 | 9 | N.S. | 9 | 8 |
| Cucumber | Copper sulfate | 85[a] | 59.3 | 78 | 66 |
| | Cuprous oxide | 93 | 50.5 | 85 | 69 |
| | None | 88 | 61.2 | 84 | 34 |
| | L.S.D. at 19 : 1 | N.S. | 4.3 | N.S. | 12 |
| Pea | Copper sulfate | 22 | 24.8 | 64 | 20 |
| | Cuprous oxide | 54 | 24.9 | 82 | 51 |
| | None | 67 | 29.2 | 78 | 18 |
| | L.S.D. at 19 : 1 | 9 | 1.8 | 7 | 11 |

[a] Sprouts blue, stunted, not geotropic.

on the other hand, is reduced or retarded in one or more cases. The column reporting results in infested soil shows that injury to the seed may be obscured by the protective action of the chemical. In the presence of a high concentration of active pathogens the cucumber and pea seeds were actually benefited by treatment. Germination of these seeds was reduced so much by pathogens that the effect of chemicals on the seed itself was not obvious.

Spinach seeds were selected as representative of the type in which germination was accelerated by copper, and pea seeds as representative of the type in which germination was retarded. Lots of 50 pea seeds and 100 spinach seeds that had been treated with cuprous oxide were germinated in Petri dishes and steamed soil in comparison with nontreated seeds. Results are presented in table 3 and figure 1. A significant acceleration of

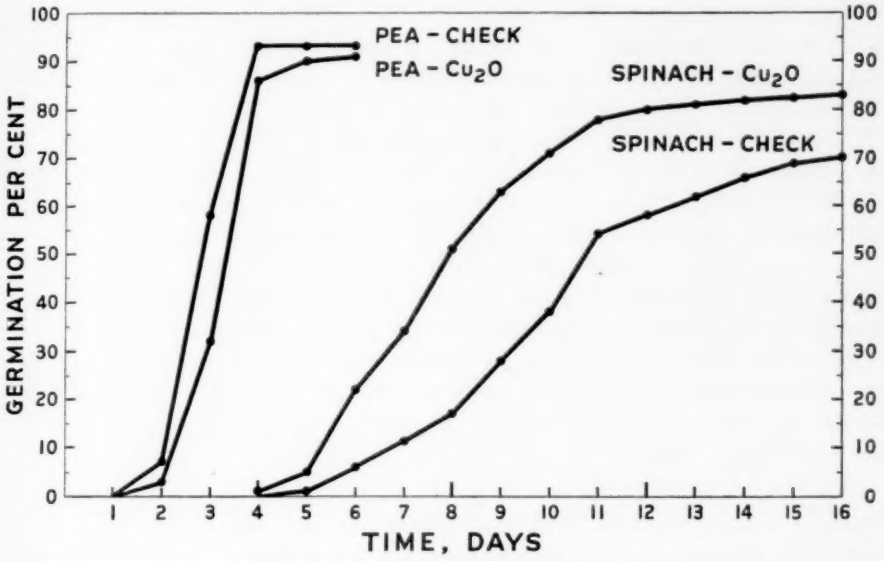


FIG. 1. The germination of seeds of pea and spinach after treatment with cuprous oxide dust.

germination of spinach seed was observed in both Petri plates and steamed soil. Pea germination was retarded in the Petri plates.

This evidence was considered adequate proof that the two kinds of seed responded differently to treatment with copper compounds. The reasons for this difference remained to be demonstrated. One possibility might be differences in the penetrability of copper ions into the seed protoplasm. Analyses of various parts of the young seedlings showed that copper had penetrated readily into treated seeds of both spinach and pea. Thus, copper ions present in the protoplasm of pea seedlings retard development while the same ions present in spinach accelerate it.

Another possible reason for the difference in response was that the fungicide altered the permeability of the seed coat to water. Seeds of peas and spinach were immersed for from one-half hour to 24 hours in distilled

TABLE 3.—*Effect of cuprous oxide dust on germination of pea and spinach seeds in the absence of pathogens. (Means of 10 samples of 50 pea and 100 spinach seeds each)*

| Kind of seed | Dosage (per cent by wt.) | Petri plate | | Steamed soil | |
|---|---|---|---|---|---|
| | | Germination (per cent) | Coeff. of velocity | Emergence (per cent) | Coeff. of velocity |
| Pea | 0.38 | 90.2 | 27.3 | 90.6 | 17.0 |
| | None | 95.0 | 30.2 | 89.4 | 17.1 |
| L.S.D. at 19:1 | | 3.3 | 1.5 | N.S. | N.S. |
| Spinach | 0.75 | 83.2 | 12.3 | 76.2 | 15.1 |
| | None | 70.1 | 9.9 | 71.0 | 15.4 |
| L.S.D. at 19:1 | | 5.8 | 1.5 | 3.3 | N.S. |

water. No significant differences could be detected in the amount of water taken up by treated and check seeds. However, if pea seeds were placed in Petri plates for four days on filter paper moistened with distilled water, the presence of cuprous oxide retarded water uptake.

The difference in water uptake demonstrated in Petri plates had not been observed in immersion tests. It seemed probable, therefore, that the utilization of water for metabolic processes had been modified by the copper and that permeability of the seed coat was not the main factor.

A quantitative measure of metabolic activity of germinating pea seeds in the absence of light can be had by dissecting out the embryo and determining its dry weight. It is assumed that the amount of dry matter converted from endosperm to embryo is a measure of the rate of metabolism. Accordingly dry weight determinations were made on four lots of 25 dissected pea embryos from the treated seedlings and an equal number from the nontreated. Results are presented in table 4.

The significantly lower fresh weight and dry weight of embryos from treated seeds is reasonable evidence that metabolic processes responsible for conversion of dry matter from endosperm to embryo were retarded in peas by the presence of copper ions. This test was not applied to spinach seedlings because the nature of the seed makes it impossible to dissect out the endosperm.

The evidence thus far indicates that copper ions present in spinach seeds accelerate germination while the same ions in peas retard germination. Moreover, the retardation in pea seedlings is related to metabolic activities

TABLE 4.—*Effect of cuprous oxide on the dry weight and water content of pea embryos from seed germinated in Petri plates. (Mean of 4 samples)*

| Treatment | Fresh weight, grams per 100 seeds | Dry weight, grams per 100 seeds | Water content, grams per gram of dry weight |
|---|---|---|---|
| Cuprous oxide | 5.41 | 0.595 | 8.1 |
| Check | 8.61 | 0.891 | 8.7 |
| L.S.D. at 19:1 | 1.16 | 0.020 | N.S. |

responsible for conversion of dry matter from endosperm to embryo. When it is considered that the energy source for all the reactions accompanying the transformation is respiration, it is logical to look next for a possible effect of copper on respiratory rate. Consequently, an investigation of the nature of enzymes used in seed respiration was made.

It has been known for some time that enzymes containing sulfhydryl groups were inactivated by copper at relatively weak concentrations. Accordingly, the nitroprusside test for sulfhydryl groups was applied to ground seeds of the seven types of vegetables used in the first tests. Positive tests were obtained with cabbage, cucumber, and pea; and negative tests with beet, eggplant, pepper, and spinach. It was thus possible to distinguish by a simple test tube method between the two groups of seeds which had been separated by germination responses.

The next question to investigate was whether copper injury to seeds of the type containing sulfhydryl groups was accompanied by destruction of the sulfhydryl groups. It was found that cucumber and pea seeds that had been treated with copper sulfate and stored ten months over calcium chloride no longer contained sulfhydryl groups. Comparable seeds that had been stored after treatment with cuprous oxide had a greatly reduced amount of sulfhydryl groups present. Nontreated seeds from the same storage maintained their sulfhydryl groups, as indicated by positive tests.

The final demonstration that copper injury to seeds was related to destruction of sulfhydryl groups was accomplished with two lots of 50 pea seeds which had been treated ten months previously with copper sulfate. These seeds would not germinate by any ordinary means and had been considered dead. When one of these lots of pea seed was placed in a Petri plate and moistened with 10 ml. of a 0.1 per cent aqueous solution of the amino acid, cysteine,[4] fifteen of the seeds germinated within two days. The water check did not germinate at all. It is obvious therefore that the inactivation of germination caused by copper can be reversed if a material rich in sulfhydryl groups is added to replace those destroyed in the seeds.

The evidence pointed to the conclusion that the effect of copper on seeds is a modification of respiratory activity. This was confirmed with ground spinach and pea seeds placed in the Fenn respirometer. Average oxygen uptake of ground spinach seed was increased by the addition of cuprous oxide, while oxygen uptake of ground pea seed was reduced. The acceleration and retardation caused by copper was thus seen to exist in ground tissue respiration in the same manner as it did in germination tests.

## DISCUSSION

The data justify the separation of the vegetable seeds studied into two groups. One contains beet, eggplant, pepper, and spinach in which germination is accelerated or increased by treatment with copper sulfate or

[4] Cysteine hydrochloride, C.P.

cuprous oxide. The other contains pea, cucumber, and cabbage in which germination is retarded or decreased by the fungicides.

In making this separation it is important to realize that relative copper concentration must be considered. It is possible to kill all seeds with a sufficiently high dosage of copper. Likewise, the use of minimum quantities of copper compounds in relatively insoluble forms, and in the presence of protective colloids or antagonistic ions is safe even on seeds which are sensitive to copper. These factors make it inevitable that in the literature it is possible to find evidence contradictory to the findings of this paper.

When fungi are present, variation in pathogenicity will also complicate results. Under the conditions of the tests reported here, fungi in the infested soil caused cucumbers to damp-off badly and, therefore, treatment with copper appeared beneficial. Peas, somewhat less susceptible to damping-off, benefited with only one of the fungicides. Cabbage, however, was relatively less susceptible to the organisms present, and the injurious effect of the copper appeared even in the presence of pathogens. There are, therefore, conditions under which a grower can afford to ignore the injurious effects of chemicals because of their relative insignificance in comparison with other factors.

In classifying seeds arbitrarily into two groups there is danger of oversimplification. It is probable that many kinds of seeds contain both copper-sensitive and copper-containing enzymes. The results reported here indicate the type of enzyme predominant in the seeds studied. If copper is present, peas might be forced to respire through other systems normally unimportant. Likewise, spinach seed in the absence of copper might develop other types of enzymes. Until further work is done on this subject any interpretation must remain tentative.

The use of a test of sulfhydryl groups in determining the mode of action of a fungicide has its limitations. Any oxidizing agent will destroy sulfhydryl groups in ground seeds in a test tube. The writer used chloranil, tetramethyl-thiuram-disulfide, chlorphenol mercury, and zinc oxide. However, the copper compounds were the only ones found to destroy the sulfhydryl groups in intact seeds. Destruction of sulfhydryl groups by copper is more rapid and complete than can be explained by ordinary oxidation.

Although the presence of enzymes containing sulfhydryl groups in peas has been proved, it should be pointed out that the presence of a copper containing enzyme in spinach seed has not been conclusively proved in this work. The inference is that one is present, and the literature to date agrees with the theory.

Because of specific injury by copper to certain types of seeds, it may be well to reconsider the interpretations placed on standard seed treatment research. Frequently a group of several compounds is used in comparison with a check, and the compound which gives the greatest number of healthy seedlings is considered the most potent fungicide. It is obvious that the kind of seed used will determine the relative rating of the chemicals.

Chloranil, for instance, is outstandingly superior to cuprous oxide for treatment of peas or beans. If the two fungicides are compared on beet or spinach seed, however, their relative superiority is reversed.


## SUMMARY

Seeds of beet, cabbage, cucumber, eggplant, pea, pepper, and spinach were germinated in Petri plates, steamed soil, and infested soil after seed treatment with copper sulfate and cuprous oxide.

The fungicides reduce or retard germination of cabbage, cucumber, and pea but increase or accelerate germination of beet, eggplant, pepper, and spinach.

Nitroprusside tests show that seeds of the three kinds susceptible to copper injury contain sulfhydryl groups, and those of the four kinds which are tolerant to copper do not.

Copper sulfate and cuprous oxide penetrate pea and cucumber seeds and destroy sulfhydryl groups normally present in them.

Moistening with cysteine solution partially restores the germinative capacity of pea seeds that have been injured by copper sulfate.

Cuprous oxide injury to pea seed is observed in a reduction of dry and fresh weights of embryos from seeds germinated in Petri plates.

Manometric measurements of oxygen uptake of ground seeds and seedlings show that suspensions of cuprous oxide reduce respiration of peas but not spinach.



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# ISOPROPANOL–SOLUBLE COMPOUNDS IN CONTROLLING STEM–END DECAY OF ORANGES

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Control of citrus decays with several water-soluble substances has recently been reported.[2] Experience with thiourea, however, focused attention on the practical importance of the toxicological properties of the compounds to be employed in stem-end-rot control because it was found that measurable amounts of this compound penetrated into the juice[2] under the conditions necessary for decay control.

In investigations to date, attention has been confined largely to water-soluble materials, presumably because of the expense of other solvents. This consideration should not necessarily preclude the trial of water-insoluble compounds. The penetration of water-insoluble compounds into the juice might be considerably less than that of water-soluble materials. It is obvious that the use of such materials, provided a suitable solvent could be found, would have the additional advantage of affording a wider range of compounds for testing. It therefore seemed advisable to change the approach to the problem by directing attention to the possibilities of water-insoluble compounds.

The experiments with water-insoluble compounds reported here cover a period from April to July, 1946, and are very limited in scope. The solvents used in preliminary tests were amyl acetate, benzene, butanol, isobutanol, butylamine, isopropanol, isopropanolamine, and combinations of these with water. Isopropanol (isopropyl alcohol) was selected for use as the solvent in the tests reported here because of its low cost and relative safety from the standpoint of rind injury.

Among the compounds tested, diphenyl sulfoxide and benzhydrol from General Chemical Company, and phenylurethane from American Chemical Paint Company, were found to be fungicidally or fungistatically active against the stem-end-rot fungi, *Diplodia natalensis* and *Phomopsis citri*. The compounds that did not prove effective in one or more of these tests were diphenyl, pinacol, diphenylmethoxy 2-methoxyethylether, tri-n-hexyl-citrate, furfurylidienemethyl isobutylketone, di-2-ethylhexyl carbonate, diphenylamine, sodium dimethyldithiocarbamate, calcium ethylene-bisdithiocarbamate, "lorol" pyridinium chloride (a mixture of 8–18 carbon atom fractions with the 12 or lauryl fraction occurring in highest percentage), "myristyl blend" pyridinium chloride (largely the $C_{14}$ fraction), and bis-(octadecyl amino) cupric chloride.

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[2] Childs, J. F. L., and E. A. Siegler. Controlling orange decay. Ind. and Eng. Chem. 38: 82–87. 1946.

The oranges available were limited to the Valencia variety by the season at which the experiments were conducted in Florida. Clipping was the principal method of harvesting, but occasional lots of fruit purchased at the packing houses had been pulled. Oranges used in a single experiment were either all clipped or all pulled, in recognition of the difference in the incidence of stem-end rot and Penicillium decay between the two types of picking. The tests conducted with this fruit were concerned primarily with control of stem-end decay. Due to light infection, a good test of control of green mold (*Penicillium digitatum*) or blue mold (*P. italicum*) was not obtained. After washing, the oranges were gassed with ethylene for approximately 40 hours before being treated. The method of treating the fruit differed slightly from previous tests in that the fruits were immersed

TABLE 1.—*The control of stem-end rot with diphenyl sulfoxide and benzhydrol in 100 per cent isopropanol, 30 seconds' immersion*

| Treatment | No. exp'ts | Total number fruit | Percentage decay | | | Total rot (per cent) |
|---|---|---|---|---|---|---|
| | | | S.E.R.[a] | Pen.[b] | Other | |
| 5 per cent Diphenyl sulfoxide | 7 | 328 | 4.3 | 6.1 | 1.8 | 12.2 |
| 5 per cent Benzhydrol | 7 | 320 | 2.5 | 1.6 | 1.3 | 5.3 |
| Check | 13 | 604 | 45.9 | 8.1 | 0.2 | 54.1 |
| 2.5 per cent Diphenyl sulfoxide | 8 | 357 | 20.4 | 1.7 | 2.2 | 24.4 |
| 2.5 per cent Benzhydrol | 7 | 327 | 14.1 | 3.7 | 0.3 | 18.0 |
| Check | 13 | 596 | 41.8 | 8.7 | 1.0 | 51.5 |
| 1.25 per cent Diphenyl sulfoxide | 5 | 223 | 23.3 | 3.1 | 2.2 | 28.7 |
| 1.25 per cent Benzhydrol | 4 | 181 | 19.3 | 2.2 | 1.7 | 23.2 |
| Check | 9 | 407 | 44.0 | 11.8 | 1.0 | 56.8 |
| 100 per cent Isopropanol | 4 | 180 | 63.3 | 10.0 | 2.8 | 76.9 |
| Check | 4 | 181 | 47.5 | 17.7 | 0.6 | 65.9 |

[a] S.E.R. signifies stem-end rot caused by *Diplodia natalensis* or *Phomopsis citri*.
[b] Pen. signifies decay caused by *Penicillium digitatum* or *P. italicum*.

in the various solutions for 30 seconds (except where noted) instead of the usual 2 to 5 seconds. They were then allowed to dry and were stored at 70° F. for three weeks, when final inspection was made.

Diphenyl sulfoxide and benzhydrol were compared at 5 per cent concentration in 100 per cent isopropanol in seven experiments totaling 1252 fruits. As shown in table 1, diphenyl sulfoxide gave good control of stem-end rot (4.3 per cent decayed) and benzhydrol gave slightly better control (2.5 per cent decayed), compared with 45.9 per cent stem-end decay in the check lots. Lower concentrations of diphenyl sulfoxide or benzhydrol in isopropanol resulted in a material decrease in control (Table 1).

Although these two compounds are only slightly soluble in water, more than 2.5 per cent of either will stay in solution in 50 per cent isopropanol. The results of four experiments with these materials at this concentration show (Table 2) 8.2 per cent stem-end rot in the fruit treated with diphenyl

TABLE 2.—*The control of stem-end rot with diphenyl sulfoxide and benzhydrol at 2.5 per cent concentration in 50 per cent isopropanol, 30 seconds' immersion*

| Treatment | No. exp'ts | Total number fruit | Percentage decay | | | Total rot (per cent) |
|---|---|---|---|---|---|---|
| | | | S.E.R.[a] | Pen.[b] | Other | |
| Diphenyl sulfoxide | 4 | 194 | 8.2 | 5.7 | 2.1 | 16.0 |
| Benzhydrol | 4 | 182 | 3.8 | 2.2 | 1.1 | 7.1 |
| Check | 7 | 324 | 41.4 | 8.6 | 1.2 | 51.2 |

[a] S.E.R. signifies stem-end rot caused by *Diplodia natalensis* or *Phomopsis citri*.
[b] Pen. signifies decay caused by *Penicillium digitatum* or *P. italicum*.

sulfoxide, 3.8 per cent in the lots treated with benzhydrol, and 41.4 per cent in the checks. Thus it appears that although a concentration of 2.5 per cent of these compounds in 50 per cent isopropanol is less effective than 5.0 per cent in 100 per cent isopropanol, it offers promise from the standpoint of practical utilization.

Phenylurethane gave results somewhat superior to the other two compounds. Ten experiments with phenylurethane at several concentrations in isopropanol (100 per cent), in which the fruits were immersed 2 to 5 seconds in the solutions, are summarized in table 3. Excellent control was obtained at 5 per cent concentration; the treated lots showed 1.0 per cent stem-end rot in contrast to 34.0 per cent in the check lots. At lower concentrations phenylurethane was not effective.

Isopropanol (50 per cent) was used as a solvent for phenylurethane (5 per cent concentration) in a series of four experiments totaling 452 fruits. The average total decay was 1.1 per cent in the treated lots and 40.2 per cent in the check lots. In very limited tests with 2.5 per cent phenylurethane in 50 per cent isopropanol the degree of control was materially reduced.

To determine the effect of the solvent on the incidence of decay, four lots (total 180 fruits) were immersed 30 seconds in isopropanol and four paired samples (total 181 fruits) were not treated. Variability of the

TABLE 3.—*The control of stem-end rot with phenylurethane in 100 per cent isopropanol, 2 to 5 seconds' immersion*

| Treatment | No. exp'ts | Total number fruit | Percentage decay | | | Total rot (per cent) |
|---|---|---|---|---|---|---|
| | | | S.E.R.[a] | Pen.[b] | Other | |
| 5 per cent Phenylurethane | 7 | 306 | 1.0 | 0.0 | 1.9 | 2.9 |
| Check | 9 | 406 | 34.0 | 6.9 | 1.5 | 42.4 |
| 2.5 per cent Phenylurethane | 6 | 286 | 7.7 | 6.6 | 0.7 | 15.0 |
| Check | 6 | 281 | 17.8 | 5.0 | 1.4 | 24.2 |
| 1.25 per cent Phenylurethane | 5 | 219 | 13.2 | 14.6 | 1.4 | 29.2 |
| Check | 6 | 257 | 17.1 | 3.5 | 1.9 | 22.6 |

[a] S.E.R. signifies stem-end rot caused by *Diplodia natalensis* or *Phomopsis citri*.
[b] Pen. signifies decay caused by *Penicillium digitatum* or *P. italicum*.

results (Table 1) was such that the differences in amount of stem-end rot or of total decay due to treatment were not statistically significant.

The possibilities of decay control with these compounds when incorporated in emulsified oil or in polishing waxes have not been investigated. However, in a single experiment, these three materials were used at 5 per cent concentration in a light summer oil and applied to oranges by the dip method. At three weeks the fruit treated with diphenyl sulfoxide showed 10.2 per cent total decay with moderate rind burn, and that treated with benzhydrol showed 11.1 per cent total decay with rather severe rind burn. Phenylurethane treatment gave 2.4 per cent total decay with only slight burns where the oil had gathered at spots of contact between fruits. There was 22.0 per cent total decay in the check lots.

The field of nonaqueous solvents for fungicides is one that has received little attention, but, judging from the above results, it merits further investigation. Some compounds that have been ineffective when used with water may prove effective in nonaqueous solvents; other effective compounds, used either in solution or dispersed in nonaqueous solvents, may prove more acceptable because of possible restricted tissue penetration.

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# WATER–CONGESTION AND FUNGUS PARASITISM

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Field observations and laboratory experiments have shown that the accumulation of excessive water in the intercellular spaces of plant tissues is relatively common under conditions favorable for its occurrence. This form of natural water-congestion arises chiefly from sources internal to the plant and hence does not include water entering tissues through wounds. The latter condition involves an additional set of factors which would complicate the problems under present consideration. The direct relation between water-congestion, host predisposition, and varietal susceptibility to certain bacterial parasites has been previously reported (2, 6). Experiments on water-congestion and fungus parasitism appeared at first more uncertain and complicated, because the conditions required for spore germination and the methods of host-penetration were not so simple as with bacterial parasites. It was shown, however, that detached, artificially water-congested unwounded leaves are readily invaded by fungi as compared to uncongested leaves under the same conditions (3).

Further progress in this study seemed to depend on inducing natural water-congestion at will in an experimental moist chamber. This is not always easily accomplished even though congestion often occurs without an intentional effort. Furthermore, since the conditions required for spore germination and infection are similar to those required for water-congestion it became necessary, for comparative purposes, to seek conditions and plants which failed to develop congestion under the same environment that favored congestion of other material. This necessitated some preliminary experiments and modifications of the moist chamber so that the soil and air environment could be varied and controlled. Detecting soils that differed in ability to yield water-congestion, and varieties responding differently in this respect was especially important. Later it was found that greenhouse-grown plants placed outdoors for a few days prior to inoculation commonly developed higher susceptibility to water-congestion and infection than did plants grown continuously in the greenhouse (4). Such variations in treatment were often applied simultaneously in order to secure comparative data on both water-congestion and infection.

The results of these studies, although not presented in detail, are believed sufficient to establish a connection between water-congestion and certain fungus diseases. Consequently they may explain a part of the frequently observed erratic behavior of some fungus diseases under natural conditions where the parasite is known to be present in abundance. An abstract of this paper has been previously published (5).

## METHOD OF INVESTIGATION

Among the basic factors involved in water-congestion are the potash content of the soil, the genetic variation in varieties of plants, and the environment under which the plants grow just prior to exposure to favorable conditions for congestion. These and other conditions may be varied in a number of different ways. For example, the age of the plants at the time of the treatments, the duration of the treatments, and the soil and air temperature at which these treatments are applied permit almost unlimited possibilities of modification in the trials. Efforts were usually made to hold as many factors constant as possible while varying only one or two factors at a time. This is often difficult with limited amounts of equipment, such as only one moist chamber, but it has nevertheless been possible to often obtain two groups of plants otherwise comparable, one of which water-congested heavily and the other of which was not visibly congested, at the same time retaining conditions suitable for infection.

The best water-congestion has been secured with sandy-loam farm soils low in potash. Fertile greenhouse compost usually fails to give visible congestion. The number of days that plants were grown outdoors in comparison to greenhouse-grown plants was often varied, but some limitations arose here, as, for example, the periods of the year when such treatment might be applied in a northern climate with highly variable temperatures. In our trials, outdoor exposures were limited to relatively short periods during the spring and fall seasons. The summer period was not adapted for such studies because of the high temperatures prevailing in the greenhouse moist chamber. The plants were exposed outdoors in an open cold frame, which could be covered at night and during rains to prevent possible wounding by storms or probable excess precipitation into the soil containers.

The moist chamber was provided with thermostatic air control and thermostatic bottom heat to provide wider differential soil and air temperatures if desired. Galvanized iron soil containers (Fig. 1) permitted more rapid increases in soil temperature when bottom heat was applied. The soil or air temperatures could be varied between about 14° and 34° C. The moisture content of the atmosphere of the chamber was the most constant factor, saturation being maintained by fine oil-nozzle atomizers directed away from the plants and so regulated as to produce a fog only sufficient to maintain a constant film of moisture on the plant surfaces.

The time of exposure in the moist chamber rarely exceeded 24 hours, frequently being 18 or as little as 4 to 6 hours, the latter often yielding infection with some pathogens. Exposures as short as one hour yielded good water-congestion with some species, although, for reasons not fully determined, 24 hours frequently failed at other times to yield visible congestion on the same species. The highly complex nature of water-congestion is obvious, but the physiological sensitivity of the plant to water-congestion is no more erratic in our experience than is the response of many plants to infection under either artificial or natural field conditions.

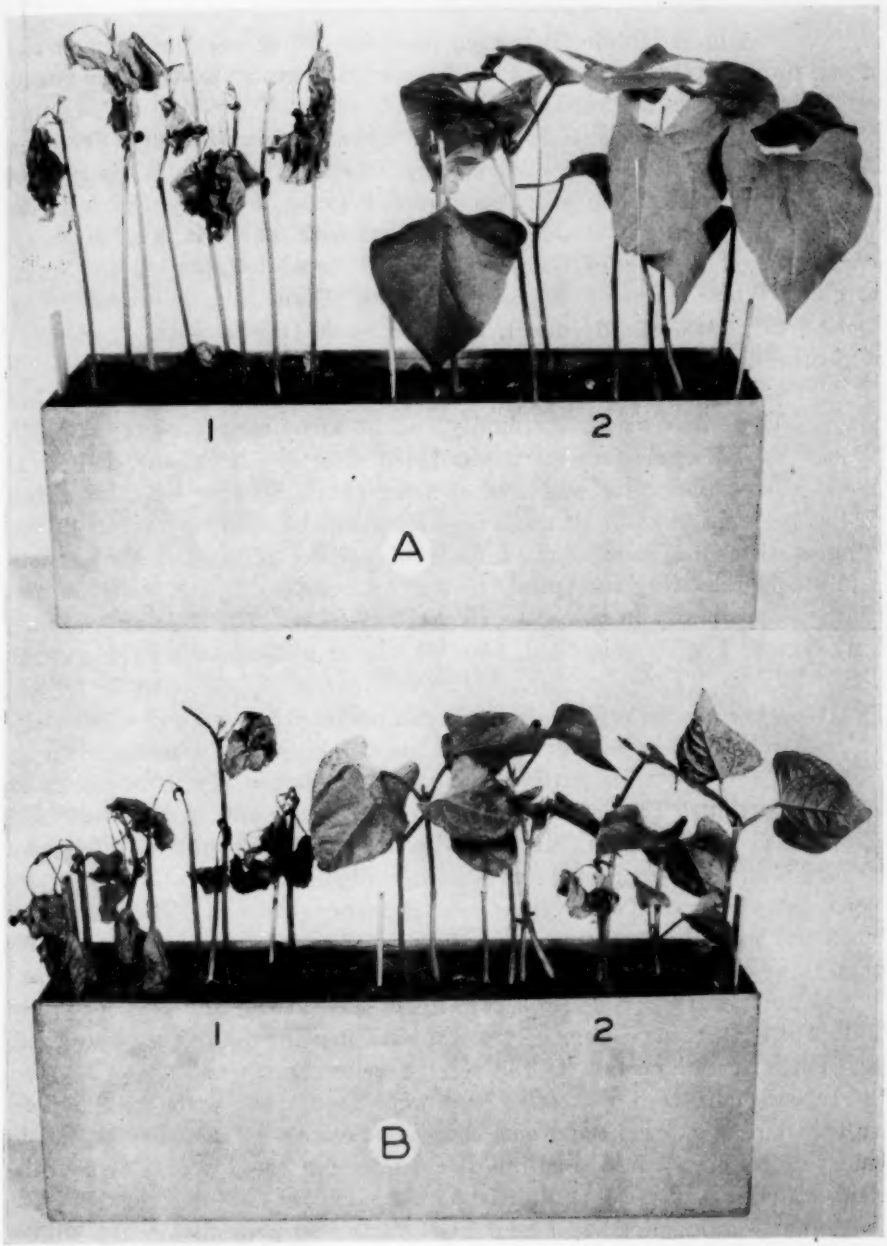


FIG. 1. Bean anthracnose on a susceptible bean variety, Bountiful (1), and on a resistant variety, Great Northern (2), grown continuously in the greenhouse (A) or placed outdoors for 3 days prior to inoculation (B). Note numerous leaf lesions in B, 2 as contrasted with the scarcity of lesions in A, 2. Metal soil containers used facilitated bottom heating. Photograph by Eugene H. Herrling.

## EXPERIMENTAL RESULTS

For this investigation, it seemed desirable to use certain common and representative types of fungi parasitic on readily grown hosts. The results on 7 such diseases are reported here.

*Bean Anthracnose caused by Colletotrichum lindemuthianum (Sacc. and Magn.) Bri. and Cav.*—The garden bean (*Phaseolus vulgaris* L.) is an ideal plant for experiments on water-congestion because of its rapid growth, the number of varieties available, and the ease with which it may be water-congested. The varieties used have included the white (Navy, Great Northern, and White Marrow), the brown or pink (Giant Stringless Green Pod, Bountiful, Wisconsin Refugee), and the black (Black Wax, Black Wax Valentine).

The experiments with beans were confined largely to the primary leaf stage. These leaves congest rapidly and heavily under the proper conditions. Visible congestion often was noted after one hour, and in 6 to 12 hours almost the entire leaf area of some plants became fully congested. It has been observed in all trials that all plants of a single variety do not congest with equal ease. Some individuals appear to be relatively resistant and others relatively susceptible to water-congestion. Bean varieties normally differ greatly in susceptibility to congestion. The resistant varieties may become heavily congested, however, under prolonged favorable conditions.

It has been noted repeatedly that bean plants grown outdoors, especially at relatively low temperatures, congest more easily than plants grown indoors. This response is partly a temperature relation as may be shown by comparing indoor plants grown at low (65–70° F.) and high (75–80° F.) greenhouse temperatures. However, as far as the amount of subsequent infection on water-congested leaves is concerned pretreatment at low greenhouse temperatures is not so effective as exposure outdoors. The beans were sown and started in the greenhouse, usually at higher average temperatures than those encountered for a 5 to 10 day period outdoors. With this method it was not always possible to secure greenhouse-grown and outdoor-grown plants of equal size or stage of growth, but often the differences were small and perhaps unimportant in the results secured.

Inoculations with *Colletotrichum* were first made with conidia from culture but in later inoculations conidia were taken from diseased stems and petioles placed in a small moist chamber for 24–48 hours for sporulation. The physiologic strain of the parasite used was not determined. The inoculum was in all cases a very heavy spore suspension applied with a DeVilbiss atomizer held a foot or more from the plants so as not to wound the epidermis or force the spores through stomatal openings. The time of inoculation varied, but usually the inoculum was applied just before the plants were set in the moist chamber. Much infection was sometimes secured on anthracnose-susceptible varieties after 12-hour exposures, and

at other times only poor infection was secured after 24-hour exposures. When water-congestion was rapid and extensive, infection was invariably heavy on leaf blades, veins, petioles, and upper and lower portions of the hypocotyl. Heavy petiole and hypocotyl infection usually resulted in early collapse of the leaves even though the number of infections of the leaf blades was relatively light. Occasionally round leaf-spot or linear vein lesions developed on the leaves only, and these sometimes occurred in the absence of good visible signs of water-congestion.

To compare amounts of infection, it was desirable to secure both good water-congestion and no congestion following the same exposure in the moist chamber. This frequently could be accomplished by utilizing selected varieties or selected soils responding differently to water-congestion, or by using outdoor and greenhouse grown plants of the same variety in the same soil. In addition it is desirable to limit the time of exposure in the moist chamber to the minimum required for infection. Many other conditions were tested, such as variations in soil moisture, soil and air temperature, light, age of plant, pre-exposure in the moist chamber prior to inoculation, etc., but the results have been less consistent than those obtained by the other methods described. The more basic factors involved in heavy infection appeared to depend upon the presence of water-congestion together with a conditioning of the epidermis or cuticle favoring penetration. These factors in turn were influenced largely by the environment in which the plants were grown a few days prior to inoculation and exposure in the moist chamber. Consequently it does not follow that all water-congested tissue is uniformly susceptible to penetration by the parasite, or that tissues which are not macroscopically congested may not be penetrated. It has been shown previously that water congestion may be so slight that it can be detected only with the aid of a microscope; yet it may be a predisposing factor to leaf penetration by pathogens (3).

Varietal differences to water-congestion are often striking, even though some varieties of beans are evidently heterozygous for this character. It is difficult to compare our results, with an undetermined physiologic strain of the parasite, with variety tests made by others in the field or greenhouse under less specific conditions as to environment or method of inoculation. No varieties of beans are said to be immune from anthracnose if tested under severe conditions, but marked differences in disease resistance have been reported under both field and greenhouse conditions. Such varietal differences have been sought for use in our tests. White Navy and Great Northern repeatedly have been noted to water-congest less readily and to be more resistant to anthracnose than Giant Stringless Green Pod, Black Wax, Bountiful, and Refugee, and yet they may become heavily congested and severely infected under favorable conditions (Fig. 1). White Marrow, although penetrated and heavily flecked with anthracnose, never has been extensively water-congested or diseased in our trials. Points of penetration of the parasite on this variety often appear to be even more numerous than

on varieties very susceptible to the disease. The congestion and penetration on Marrow appears to be limited largely to the leaf veins, where incipient superficial lesions develop in especially large numbers and eventually coalesce into larger discolored streak areas, but intensive necrosis is rare.

The conclusions relative to bean anthracnose are based on 56 series of inoculations with *Colletotrichum lindemuthianum*, and, although the results have varied greatly with the method of treatment, all show that host-predis-

TABLE 1.—*The relation of water-congestion to development of anthracnose on bean plants grown continuously in the greenhouse or given an outdoor pretreatment prior to inoculation without wounding*

| Exp. | Incubation | | Pretreatment | | Host variety | Early signs of infection[a] on | | | | Final development of disease[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hours | Temp., °C. | Days | Place | | Visible water-congestion[a] | Leaf-blades | Veins | Petioles | Stems |
| 1 | 24 | 20–23 | 7 | Outdoors | Bountiful | ++++ | + | + | + | + | ++++ |
| | | | | | White Marrow | + | 0 | + | 0 | 0 | + |
| | | | | Indoors | Bountiful | 0 | 0 | 0 | 0 | 0 | ++ |
| | | | | | White Marrow | 0 | 0 | 0 | 0 | 0 | + |
| 2 | 24 | 18–20 | 7 | Outdoors | Bountiful | ++ | + | + | + | + | ++++ |
| | | | | | White Marrow | + | 0 | + | 0 | 0 | + |
| | | | | Indoors | Bountiful | + | 0 | + | + | 0 | ++ |
| | | | | | White Marrow | + | 0 | 0 | 0 | 0 | + |
| 3 | 24 | 21–25 | 7 | Outdoors | Black Wax | ++ | + | + | + | + | ++++ |
| | | | | | White Marrow | + | 0 | + | 0 | 0 | + |
| | | | | Indoors | Black Wax | 0 | + | + | + | 0 | ++ |
| | | | | | White Marrow | 0 | 0 | + | 0 | 0 | + |
| 4 | 15 | 22–24 | 3 | Outdoors | Bountiful | ++ | + | + | + | + | ++++ |
| | | | | | Great Northern | ++++ | + | + | 0 | 0 | +++ |
| | | | | Indoors | Bountiful | ++ | + | + | + | + | ++ |
| | | | | | Great Northern | + | + | 0 | 0 | 0 | + |
| 5 | 20 | 19–25 | 8 | Outdoors | Black Valentine | ++ | + | + | + | + | ++++ |
| | | | | | Great Northern | + | + | + | + | 0 | ++ |
| | | | | Indoors | Black Valentine | ++ | + | + | + | + | ++ |
| | | | | | Great Northern | + | 0 | 0 | 0 | 0 | + |

[a] Amount of water-congestion, infection, or disease: 0 = none visible; + = small; ++ = moderate; +++ = heavy; ++++ = very heavy.

position and relative susceptibility of bean varieties to anthracnose may be altered by conditions which bring about variation in water-congestion (Table 1). This conclusion does not contradict previous observations of others that such conditions as stage of maturity and other gross factors are involved in anthracnose infection; it rather offers a possible explanation for such behavior. Not only the stage of maturity but the particular plant organ attacked may influence the results and interpretation of experiments designed to vary host-predisposition. Although varietal variation to anthrac-

nose infection may be explained on the basis of water-congestion, it seems likely that still other factors are involved in the later stages of disease development.

*Potato Late-Blight* (*Phytophthora infestans* (Mont.) *de B.*).—Visible signs of water-congestion rarely were obtained on the leaves of potato in the moist chamber, although it can be demonstrated to be present by submerging the leaves in rose bengal dye. The potato leaf may be congested readily by artificial water pressure and, in common with many other species of plants, is able to take up 20 per cent[1] or more of its original weight as congestive water. In the potato it appears that microscopic or invisible amounts of water-congestion in tissues are sufficient to favor infection by *Phytophthora;* but, without any water-congestion, infection evidently does not occur.

Following the usual method of inoculation, little difficulty was experienced in securing severe expressions of the disease which eventually resulted in the death of the plants. Varying the ordinary environmental factors under which the plants were grown prior to inoculation appeared to have little effect on subsequent disease development. Varieties reported to have some resistance (Sebago, Sequoia) collapsed as rapidly as Triumph, Red Warba, Chippewa, and Katahdin varieties (Table 2). It then became evident that an exposure of 24 hours in the moist chamber was so favorable to infection that all varieties become about equally infected. Furthermore, it also was obvious that conclusions on penetration should be based only on the number of original points of infection and not on later stages of blight development. Potato late-blight, even in the greenhouse, continues to spread into new tissues, with the result that the entire plant may succumb from relatively few points of infection.

With the potato, therefore, it seemed desirable to lower the time of exposure sufficiently to prevent such heavy infections as tend to mask both variations in host-predisposition and relative varietal resistance to disease. When the time of exposure in the moist chamber was lowered to 6, 9, or 12 hours, other conditions being favorable, the number of necrotic lesions produced at the points of infection were fairly proportional to the relative resistance and susceptibility of the varieties used. Following short exposures in the moist chamber, plants grown outdoors 5 to 10 days prior to inoculation were more predisposed to infection than greenhouse-grown plants, even though visible signs of water-congestion were not evident. This response nevertheless supports the conclusion that congestion was present, though not visible. The significance of water-congestion to infection of potato with *Phytophthora* is regarded, however, as being more dependent on interpretation than is the case of bean anthracnose.

*Tomato Late-Blight* (*Phytophthora infestans* (Mont.) *de B.*).—The behavior of *Phytophthora* on tomatoes is more easily interpreted on the water-congestion basis. No difficulty was experienced in securing heavily infec-

[1] The data dealing with the amount of water-congestion, based on percentage increase in weight of plant tissue, will be presented in a separate paper.

tions with the potato strain of the fungus. The tomato congests easily; the number of infection points may be far more numerous than on the potato with the same inoculum, but the subsequent development of the disease in the greenhouse is more limited to the original infected areas and the host hence tends to recover from heavy infections more readily than is the case with potato.

TABLE 2.—*The relation of water-congestion to infection and disease development in 4 rusts and 2 late blight diseases on plants grown indoors or given a pretreatment outdoors prior to inoculation without wounding*

| Disease | Incubation | | Pretreatment | | Host variety | Visible water-congestion[a] | Early signs of infection[a] | Final development of disease[a] |
|---|---|---|---|---|---|---|---|---|
| | Hours | Temp., °C. | Days | Location | | | | |
| Wheat stem rust | 24 | 19–21 | 6 | Outdoors | Marquis | ++++ | ++ | +++ |
| | | | | | Henry | ++++ | + | + |
| | | | | Indoors | Marquis | + | + | ++ |
| | | | | | Henry | ++ | 0 | + |
| Oat leaf-rust | 12 | 19–21 | 8 | Outdoors | States Pride | ++ | + | +++ |
| | | | | | Vicland | + | ++ | +++ |
| | | | | Indoors | States Pride | 0 | + | ++ |
| | | | | | Vicland | 0 | + | +++ |
| Corn rust | 6 | 18–20 | 5 | Outdoors | Golden Bantam (sweet) | +++ | ++ | ++++ |
| | | | | | Golden Glow (field) | + | + | +++ |
| | | | | Indoors | Golden Bantam | 0 | + | + |
| | | | | | Golden Glow | 0 | + | + |
| Potato late-blight | 6 | 18–23 | 7 | Outdoors | Triumph | 0 | + | ++ |
| | | | | | Sebago | 0 | ++ | +++ |
| | | | | Indoors | Triumph | 0 | 0 | + |
| | | | | | Sebago | 0 | 0 | + |
| Tomato late-blight | 7 | 20–22 | 10 | Outdoors | Marglobe | ++ | ++ | ++++ |
| | | | | Indoors | Marglobe | 0 | + | + |
| Sunflower rust | 12 | 18–22 | 14 | Outdoors | Mammoth Russian | 0 | ++ | ++++ |
| | | | | Indoors | Mammoth Russian | 0 | + | ++ |

[a] Amount of water-congestion, infection, or disease: 0 = none visible; + = small; ++ = moderate; +++ = heavy; ++++ = very heavy.

As a result of this behavior, it is frequently possible to observe first the marginal and angular water-congested leaf-areas, and to note subsequently their relation to the points of infection, and to the limits of progress of the disease in these areas. When the thin tomato leaves return to normal water relations, the progress of the invasion appears to be sharply halted, as contrasted to the reaction on the potato. The results with *Phytophthora* on

tomato (Table 2) are in line with those secured with bean anthracnose, and are more readily demonstrated and interpreted than the results with potato.

*Barley Leaf-Blotch* (*Helminthosporium sativum P. K. and B.*).—Among 35 species of plants tested, including mostly common crop plants, barley was the most susceptible to natural water-congestion. It was more difficult to congest greenhouse grown barley plants at will during the winter months, and typical or heavy infections on the leaves were not obtained from inoculations with *Helminthosporium*, although good varietal differences in stem infections (foot-rot) were obvious. It was not certain, however, that the spores from the cultures possessed an equal degree of infectivity. These and other factors may account for the relatively inconsistent results secured with leaf-blotch of barley. In two out of several trials, however, obvious correlations between water-congestion and infection occurred in outdoor and greenhouse grown plants, and between resistant (Oderbrucker Wis. Pedigree 5) and susceptible (Wis. Barbless Pedigree 38) barleys.

*Leaf-Rust of Oats* (*Puccinia coronata Corda*).—Oats are less susceptible to water-congestion than some other grains, but little difficulty was experienced in securing good congestion under favorable conditions. Proper conditions again involved chiefly the selection of a suitable soil and pre-exposure of plants outdoors for 5–10 days prior to exposure in the moist chamber. As a rule, the tests were conducted in the early seedling stage. The varieties most frequently used were States Pride (rust-susceptible) and Vicland (rust-resistant). The strain of leaf rust originally used was collected from a local field of the States Pride variety. An abundance of young spores from previously infected plants was usually available for inoculations. Inoculations were made with water suspensions from an atomizer, since this method appeared to offer better possibilities than spore dusting for a uniform distribution of spores over the leaf surfaces. The usual period allowed in the moist chamber was 12, 18, or 24 hours.

Flecks and pustules were always more numerous on plants that had been exposed outdoors than on plants continuously indoors. Frequently excellent infection was secured on outdoor plants and none on indoor plants (Fig. 2). The number of flecks or pustules was not necessarily proportional to the amount of water-congestion, however; and, as in the case of bean anthracnose, it is clear that other factors in the epidermal layer or the cuticle, especially the stomatal position, are highly important to infection when associated with water-congestion. It seems fairly certain from several series of trials that little or no infection from leaf-rust of oats developed in the absence of water-congestion.

Of special interest in the trials with oats was the relative infection secured on normally resistant and susceptible varieties when the pretreatments were varied before inoculation and confinement in the moist chamber. Commonly, States Pride (susceptible) congested earlier and yielded heavier infection than Vicland (resistant). In some trials, however, Vicland appeared to be more susceptible than States Pride as far as penetration

and early infection were concerned (Table 2). Nevertheless, Vicland rarely yielded more or larger pustules than States Pride. Vicland was obviously more resistant to subsequent progress of the fungus, indicating that more than one factor is concerned with disease resistance to leaf-rust in oats. It

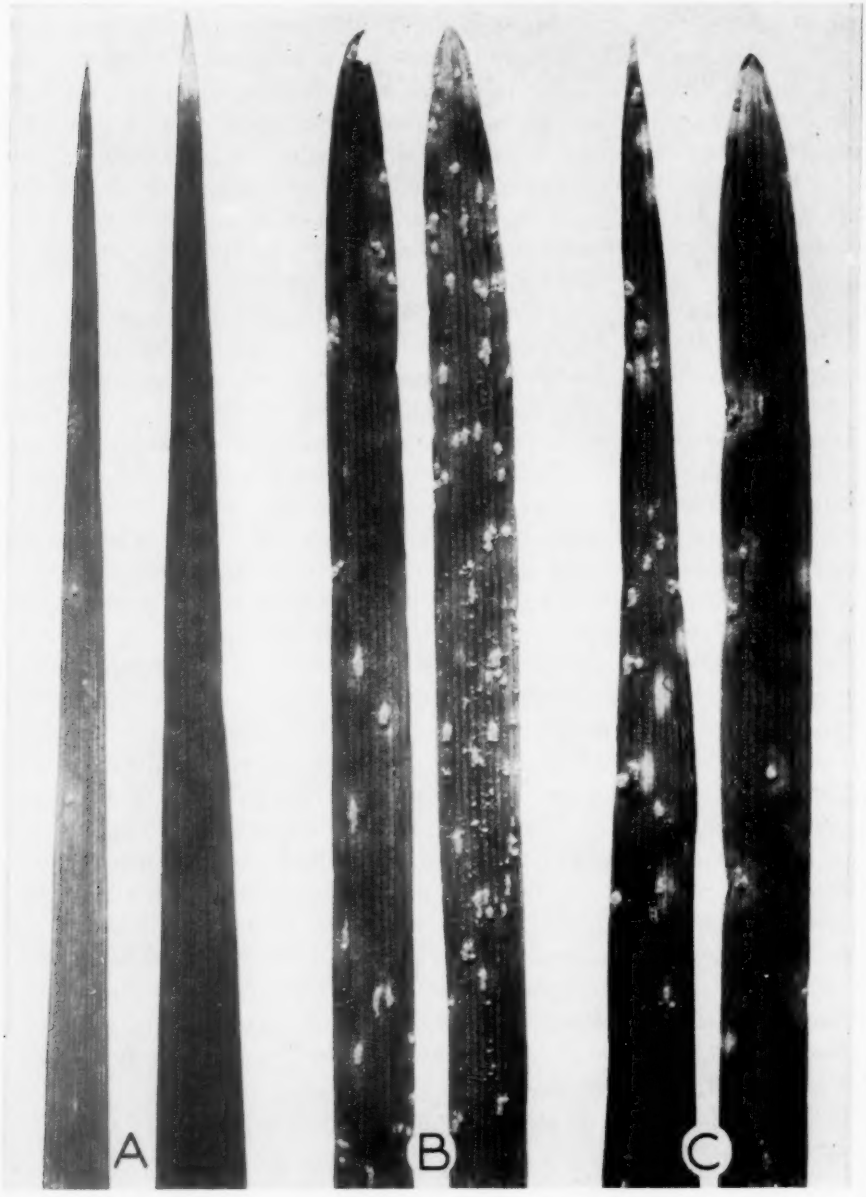


FIG. 2. Leaf rust on a susceptible variety of oats, States Pride, grown continuously in the greenhouse (A) or grown outdoors for 7 days prior to inoculation (B), and on a resistant variety of oats, Vicland, grown outdoors for 7 days prior to inoculation (C). Photograph by Eugene H. Herrling.

is suggested that the intercellular spaces may be smaller in the resistant variety, thus they may retain less congestive-water and check the progress of the parasite earlier than in the susceptible variety.

*Wheat Stem Rust* (*Puccinia graminis Pers.*).—The strain of wheat rust used was not determined, but the original inoculum was secured from a nursery heavily infected with stem rust after artificial inoculation with race 56 of *Puccinia graminis tritici*, and the purity of the rust was presumably retained. However, the experimental results are based only on symptoms secured on either young seedlings or leaves of older plants prior to the heading stage. Four varieties of wheat were used; namely, Henry and a durum variety (resistant) and Progress and Marquis (susceptible). Henry and Marquis were most frequently compared.

As in the case of oats, water-congestion was regularly more pronounced on wheat plants grown outdoors some days prior to inoculation (Table 2). The amount of rust infection on spray-inoculated plants was, as a rule, fairly proportional to the amount of congestion. Occasionally, the amount of congestion on outdoor plants of the Henry variety (resistant) was more conspicuous than on Marquis, although the reverse was the case on indoor plants. In one trial this behavior was closely related to the amount of subsequent infection and pustule development, so that outdoor plants of the resistant variety appeared relatively susceptible and the indoor plants of the same variety appeared relatively resistant.

During the winter months when plants could not be grown outdoors prior to inoculation, the variation of other environmental conditions (including soil type, soil moisture, soil and air temperature, pre-exposure in the moist chamber prior to inoculation and other treatments) yielded no convincing differences in amount of infection. It was not until outdoor pretreatment could be combined with soil differences that significant variations in infection were obtained. It seems very likely that in our trials some of the erratic behavior toward infection in the presence of water-congestion is attributable to varietal differences in stomatal behavior as described by Hart (1).

In some of the trials with wheat, natural infection with wheat mildew (*Erysiphe graminis* D. C.) developed. This type of pathogen, which commonly develops under environmental conditions apparently not favorable to water-congestion, was nevertheless increased in severity by water-congestion of the host tissues.

*Corn Rust* (*Puccinia sorghi Schw.*).—Corn seedlings usually water-congest easily and quickly in a moist chamber, as they often do on greenhouse benches or in the field when the humidity is high. We have not noted as yet any striking or consistent differences in varieties in this respect, but only four have been grown in our tests. For the most part, Golden Glow (field corn) and Golden Bantam and Stowell's Evergreen (sweet corn) were used. Trials were made with some strains of sweet corn that are believed to vary in rust resistance, but no significant differences were found.

The corn rust originally was collected from the field and an abundance of inoculum was available thereafter from greenhouse plants. Good infections may be obtained with atomized spores after relatively short exposures (4–8 hours) in the moist chamber, although longer exposures often yielded heavier infection. A variety of pretreatments with greenhouse grown plants failed to yield significant variations in either the amount of water-congestion or the amount of infection.

It was not until the spring months, when normal corn plants could be grown outdoors, that host-predisposition to rust could be influenced to a significant degree. Corn plants grown outdoors (at a favorable temperature) for 5–10 days, and exposed in the moist chamber for a period as short as 6 hours at 18° C., congested very heavily and yielded 3 times as many pustules as greenhouse grown plants in one trial (Table 2).

By reducing exposure in the moist chamber to a time that was minimum for congestion and penetration in a susceptible variety but insufficient for congestion in a resistant variety, it was possible to obtain differences in rust infection on varieties of corn. Because field conditions seldom coincide with this experimental procedure, there may appear to be no differences in field reactions of these sweet corn strains to rust. Good infection has been obtained on corn in the presence of very little visible water-congestion, and it cannot be overemphasized that the amount of visible water-congestion alone is not necessarily correlated with the amount of fungus penetration.

*Sunflower Rust* (*Puccinia helianthi Schw.*).—Only one variety of sunflower (*Helianthus annuus* L.) was used. The fungus was obtained from a natural infection of plants in the field. Infection was so regular and extensive under the greenhouse conditions of the experiments that the period of exposure in the moist chamber was gradually reduced to 4 or 5 hours in order to secure significant variation in the development of the disease. However, visible water-congestion was not obtainable either with long exposure in the moist chamber or with widely varying pretreatments. The dye bath method of testing for the presence or absence of water-congestion also yielded irregular results. Sunflower leaves, however, may be easily and heavily congested with artificial water pressure. In general, it appeared that when moisture was maintained on the leaf surface for only 1 to 2 hours beyond the time required for spore germination, infection occurred without regard to any apparent predisposing condition of the host. Nevertheless, the variability of different leaves on the same plant and of different parts of the same leaf suggested that some undetermined factor was concerned with infection. It is probable that if varieties of sunflower differing in susceptibility had been available, further evidence on this point might have been obtained.

When sunflower plants grown outdoors for 5–10 days were compared to indoor grown plants, and the period of exposure in the moist chamber was sufficiently reduced, marked differences in amounts of infection were obtained (Table 2). The results sufficiently approached those secured with

other diseases to show that the outdoor influence on host-predisposition was not entirely absent. On the basis of these comparable trials, there is reason to believe that some excess water is present in the intercellular spaces when infection takes place.

## DISCUSSION

Natural water-congestion plays a fundamental rôle in infection with certain bacterial diseases, especially with tobacco wildfire (2, 6). With artificial water-congestion of detached leaves of various species of plants, a probable relationship to infection with certain fungi was also indicated (3). Many field observations have supported the preliminary laboratory studies, but actual experimental evidence seemed to be required to secure further information. In this investigation, the chief difficulty has been to obtain differential degrees of water-congestion at will while maintaining other conditions identically favorable for infection by the pathogen. Unfortunately, water-congestion is induced by the same conditions that favor infection; consequently, some pretreatment must be relied upon to delay or prevent congestion under conditions which permit infection. Thus far, this has been frequently partially accomplished. Complete freedom from congestion is not readily determined.

Many other phases of the problem have arisen during the course of this study. It is clear that water-congestion alone, in the absence of wounds, is not always sufficient to permit penetration of a pathogen into host tissues. With the bacteria, the stomatal position and the condition and amount of the waxy cuticle are obviously associated factors. Many of the fungi with which we have worked are known to penetrate through the stomata, but with the bean anthracnose fungus it has been generally accepted that direct penetration of the epidermal cells by germ tubes from an appressorium is the normal method of infection. It is possible that both direct and stomatal penetration of the bean leaf may occur but that the relative frequency of each may depend upon environmental conditions. From results secured with the bean anthracnose fungus in the present investigation, it is believed that the method of penetration is partly stomatal because of the short periods of exposure required for infection in a favorable environment. Some of these periods apparently were shorter than those previously reported necessary for spore germination alone. Although our knowledge of this subject is still meager, it obviously is important that the optimal conditions for host penetration should be known before the predominating type of invasion can be satisfactorily determined. For present purposes we may need to assume only that the parasite enters the host through the path of least resistance. When the tissues are unwounded and water-congested, it seems likely that this path is through the stomatal openings, especially when the appressoria lie immediately above.

When all the factors of host-predisposition and variation in resistance

to disease are taken into account, as well as the influence of environmental conditions on the parasite itself, it is not difficult to understand the frequently observed erratic behavior of plant pathogens. The conclusion that water-congestion plays an important part in this behavior with some fungus diseases, as it does for certain bacterial diseases, seems to be justified.

## SUMMARY

Natural water-congestion in many species of plants may be produced experimentally under favorable moist-chamber conditions. The plants water-congest most readily if grown outdoors in sandy or sandy-loam soils low in potash. For experimental purposes, it usually suffices to expose greenhouse grown seedlings to outdoor environment for 5–10 days prior to transfer to the moist chamber. With other soil types, or with plants not exposed outdoors, many species or varieties water-congest only very slowly. Thus, by limiting the time of exposure of inoculated plants in the moist chamber, it was possible to secure both heavily water-congested plants and plants not visibly congested during the same period of incubation.

Marked differences in rate and amount of visible water-congestion occurred between varieties and sometimes between individual plants of the same variety. Water-congestion developed in some species in as little time as one hour. In other species, *e.g.*, the potato and the sunflower, visible natural water-congestion was not obtained in the moist chamber.

Plants of several species, following growth and pretreatment under varying conditions, including those described, were inoculated with representative fungus pathogens and incubated in the moist chamber under conditions favorable for water-congestion. Results are reported on several fungus diseases: bean anthracnose, potato late-blight, tomato late-blight, leaf-blotch of barley, leaf-rust of oats, wheat stem rust, corn rust, and sunflower rust.

It was found that water-congested plants of the same variety were more predisposed to infection by certain fungi than plants not water-congested. Varieties susceptible to disease congested more easily as a rule than varieties resistant to the fungus parasites in question. The amount of fungus penetration and infection was not always correlated with the amount of water-congestion. Other conditions not definitely determined, but favored by outdoor exposures as contrasted to continuous greenhouse culture, were involved.

The varied conditions of the experiments were such that the genetic resistance of some varieties often was obscured by heavy infection and the resistant and susceptible varietal characters apparently reversed.

Although potato and sunflower yielded no visible water-congestion in the moist chamber in these particular trials, good infection with late-blight and rust, respectively, was secured on both indoor and outdoor plants. More infection was secured on outdoor grown plants, and the results are believed to be associated with macroscopically invisible amounts of water-congestion.

Such a form of slight water-congestion has been demonstrated microscopically, as well as by the use of dyes and by comparative weights.

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## PHYTOPATHOLOGICAL NOTES

*A Rapid Method of Examining Wheat Heads for Bunt Infection.*[1]—Bunt of wheat (*Tilletia foetida* (Wallr.) Liro, and *T. caries* (DC.) Tul.) is usually detected in the field by a visual inspection of the heads supplemented, whenever necessary, by an examination of individual spikelets for bunt balls. If the diagnosis is carried out by one who is familiar with the symptoms of bunt infection the presence or absence of bunted grains can be accurately determined. When the purpose of the examination is limited to the collection of bunted heads or where it is desired to determine only approximately the degree of bunt infection a visual examination of heads will usually suffice. It frequently happens, however, that only one or two spikelets in a head are bunted; so that, if the purpose of the examination is to determine accurately the degree of infection, it becomes necessary to inspect each individual spikelet. This requires considerable time and labor, especially in experiments involving the inoculation of a number of wheat varieties with several physiologic races of bunt. In such instances, the task of examining each individual spikelet in several thousands of heads places a definite limitation on the amount of experimental work that can be undertaken. To facilitate the detection of bunt some workers have tried clipping the individual florets of the heads whereas others have resorted to threshing each head individually and then examining the grain for the presence of bunt balls, but even these devices require considerable time.

The present note describes a method of detection based on the observation that bunted heads of ripening wheat are more conspicuous immediately following a drenching rain. The method is simple and has proved to be rapid and accurate.

The grain to be examined is harvested when the majority of the heads are ripe. The percentage bunt infection may be determined at once, or the grain may be stored and examined for infection at a later date. Immediately prior to examination the heads are soaked in water. Soaking may be done in either of two ways: (1) By immersing the heads in water at or near the boiling point and leaving them to soak overnight. (2) By immersing the heads for about 24 hours in water (at about room temperature) to which a wetting agent has been added.

The examination for bunt is carried out in a good light while the heads are still wet. The contrast in bunted heads before and after soaking in water is very striking (Fig. 1). When soaked the bunt balls become swollen and much darker. They can usually be recognized either through or between the glumes which have become semi-transparent and spread apart.

It should be borne in mind that if the heads are soaked in hot water the bunt spores will be killed and so rendered useless as a source of inoculum for smut experiments. Hot water should be used, therefore, only when a wetting agent is not available or when the spores are not required for inoculum.

[1] Contribution No. 897, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

In one experiment in which 20 varieties of spring wheat (15 of *Triticum vulgare* and 5 of *T. durum*) were inoculated separately with *Tilletia foetida* and *T. caries*, a total of 68,000 heads were examined for bunt infection. When the heads were examined in the dry condition, 10,147 (14.9 per cent) were classified as infected. When examined after soaking in water infection was found to be present in 12,558 heads (18.5 per cent). From these results it will be apparent that an examination of the heads after soaking may be expected to give more accurate bunt counts.

In certain varieties it was frequently found that only one or two kernels in a head had become infected, and occasionally infection was restricted to

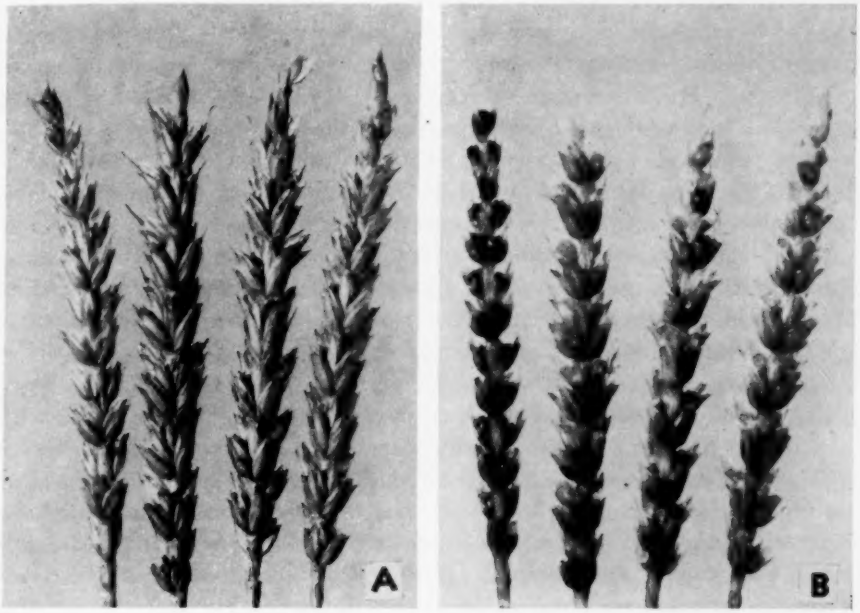


FIG. 1. The effect of water-soaking of wheat heads on the visibility of bunt infection. A. Smutted heads of Marquis wheat showing practically no symptoms of bunt before soaking in water with the exception of the head on the extreme left. B. Same heads as in A after soaking for 24 hours in water containing a wetting agent. Note darkening effect caused by bunt balls showing through water-soaked glumes.

only a portion of the kernel. In such instances it was extremely difficult to detect the infection by a visual inspection of the dry heads whereas, after soaking, the infected kernels were, in most instances, readily apparent. Partially bunted heads were encountered frequently in the variety Regent following inoculation with certain physiologic races of bunt. In one experiment necessitating the examination of 3,400 heads of this variety, bunt infection could be detected in only 34 (1.0 per cent) when the heads, but not the individual spikelets, were examined in the dry condition. After soaking in water, 100 (2.9 per cent) of the 3,400 heads were found to be infected.

It is believed that the method just described will be found to be particularly useful where a large number of heads has to be examined for bunt

infection. In practice it has been found that as many as 2,500 heads of wheat can be examined by one person in an hour.—W. POPP, Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba, Canada.

*Advantages of Natural Media and Environments in the Culture of Fungi.*—When a fungus is encountered which grows or fruits well in nature but only poorly or not at all on artificial media in the laboratory it has seemed logical to resort to a natural medium, and to a fluctuating environment where such factors as range and periodicity of temperature, of light intensity, and of relative humidity are varied. It would seem that the culture of fungi might be handled in general to greater advantage were more consideration given to how and under what conditions fungi develop in nature and to what they require in the way of substrates and environments.

An example of one of the natural media used effectively for both the isolation and sporulation of certain fungi is pea-straw agar prepared from water agar and chopped pea straw which has been sterilized by fumigation.[1] When *Ascochyta pinodes* or *A. pinodella* is cultured on this pea-straw agar the hyphae grow into the medium and develop an abundance of dark, characteristic chlamydospores about and on the pieces of straw distributed through it, while pycnidia may appear on the surface of the medium. Where field surveys are to be made plates of pea-straw agar are taken into the field and even the underground parts of plants may be placed directly on the medium after shaking off the free soil. Since very little aerial mycelium develops on this agar the Ascochyta species may be identified usually in a week's time by direct microscopic examination of the plates. *Verticillium albo-atrum* is readily cultured from diseased tissues on this medium, simply by whittling small pieces of the infected tissue on the pea-straw agar. No sterilization of the plant part is required nor do laboratory contaminants which usually over-run potato-dextrose-agar plates interfere seriously with the recovery of *Verticillium*. The Verticillium hyphae grow into the medium and produce micro-sclerotia abundantly about and on the bits of straw, while typical conidiophores and conidia appear on the surface of the agar. Species of such genera as *Phytophthora*, *Pythium*, and *Rhizoctonia* also develop submerged hyphal growth in a medium of this sort and are easily recovered in pure culture by hyphal-tip transfer of the submerged mycelium from the original tissue platings of diseased material. *Alternaria solani*, which sporulates poorly or not at all on potato-dextrose agar, sporulates abundantly within a week after this medium is flooded with inoculum. A species of *Glocosporium* which also did not sporulate on the usual artificial media did so profusely on the pea-straw agar.

It has been evident that the kind of organic matter used as a medium need not be that on which the fungus is found in nature. For example, perithecia of *Mycosphaerella pinodes* have been produced abundantly on the

[1] Hansen, H. N., and William C. Snyder. Gaseous sterilization of biological materials for use as culture media. Phytopath. 37: 369–371. 1947.

wheat straw in a wheat-straw agar (fumigated wheat straw plus water agar) under proper environmental conditions, almost to the exclusion of either aerial mycelium or pycnidia.

These are only two examples of many natural media which have proved useful. Above and below-ground parts of plants, and scale and other insects have been used effectively for the culture of fungi after fumigation and placement on or in water agar, depending upon the substrate preferred and the purpose for which the medium is to be used.

The influence of a natural environment on the development of fungi is illustrated by still other examples. *Centrospora acerina* sporulates profusely on agar media outside a north window but not at all inside this window. The same is true of the imperfect stage of *Botryosphaeria ribis* and of *Mycosphaerella brassicicola*. A similar experience has recently been reported by Houston and Oswald,[2] who found that *Helminthosporium gramineum* sporulated rapidly and abundantly in an outdoor environment but not indoors.

The importance of both light and temperature in relation to the sporulation of fungi is well known but the results obtained here indicate further that diurnal fluctuation in both of these factors influences favorably the normal fruiting of fungi. Even the lack of fluctuation in humidity results in abnormal completion of spore discharge in some fungi. Perithecia of *Hypomyces solani*, *Gibberella roseum*, and *Mycosphaerella pinodes*, for example, exude their ascospores in wet masses or tendrils during periods of continuously high humidity but eject them forcibly into the air when periods of low humidity alternate with periods of high humidity.

Besides these environmental influences are still other factors for consideration such as the long cyclic fluctuations in the seasonal environment, and the direction of this cycle, whether, for instance, the cycle is entering a period of longer days with shorter nights or vice versa. That even these fluctuations have a bearing on the growth and sporulation of fungi in culture is evident from the relative speed, profusion, and quality of fruiting of various fungi when grown at different times of the year.—WILLIAM C. SNYDER and H. N. HANSEN, Division of Plant Pathology, University of California, Berkeley.

*Powdery Mildew on Cherry Fruit in Washington.*—During the latter part of July, 1944, the writer's attention was called to a disease affecting sweet cherry fruits harvested from orchards located in the upper portion of Squilchuck Canyon, near Wenatchee, Washington. An examination of the diseased specimens revealed the presence of powdery mildew hyphae and conidia. In a subsequent visit to the infected orchards the perefet stage of the fungus was found and later identified as *Podosphaera oxyacanthae* (DC.) D By.

[2] Houston, B. R., and J. W. Oswald. The effect of light and temperature on conidium production by *Helminthosporium gramineum* in culture. Phytopath. **36**: 1049–1055. 1946.

Although powdery mildew for many years has been known to occur on the leaves and shoots of the cherry in various sections of the United States,[1, 2] a review of the readily available literature has failed to uncover any mention of fruit invasion. In the vicinity of Wenatchee the disease occasionally has been observed on leaves and shoots, but here also no previous records of fruit infection have been found.

The symptoms of the disease on fruits of sweet cherry are rather striking, and are distinctly different from those of any other disease known to attack

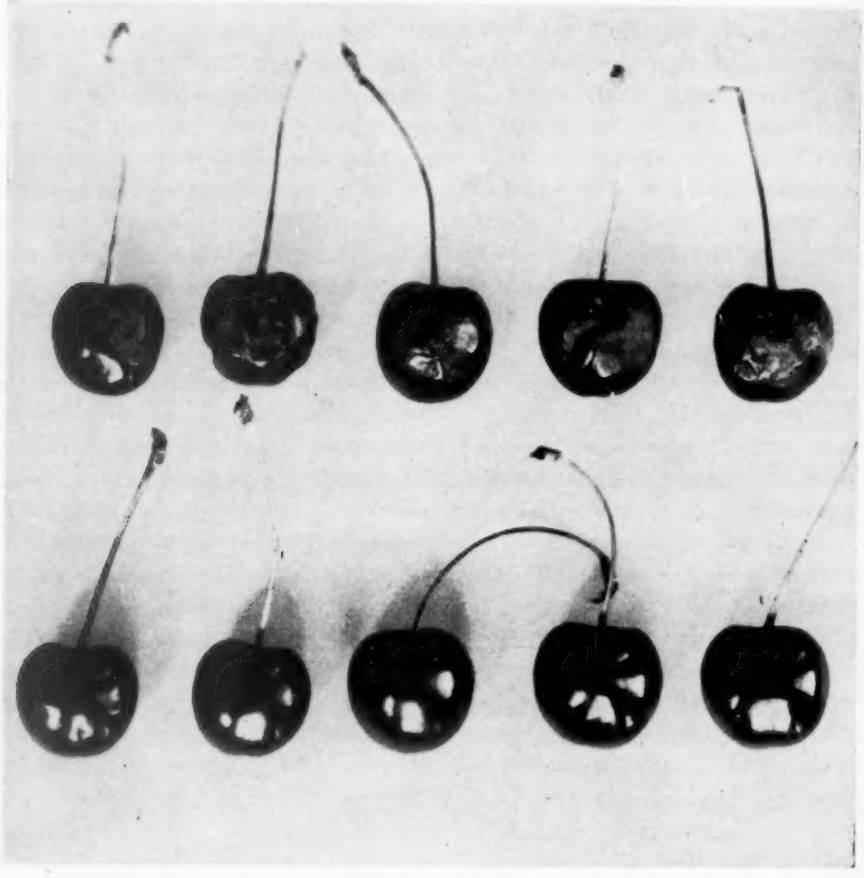

FIG. 1. Powdery mildew on fruits of the Bing cherry: Upper fruits diseased, lower ones healthy. Note the shriveled and dull appearance of the infected fruits as contrasted to the high gloss of the normal cherries.

cherries. The invaded areas are circular, or more frequently irregular, due to the coalescence of two or more infections (Fig. 1). A thin, white mantle of hyphae, which radiate from the center of young lesions, covers the infected

[1] Galloway, B. T. The powdery mildew of the cherry. U. S. Agr. Comm. Rept. 1888: 352–357. 1889.

[2] Stewart, Vern B. Some important leaf diseases of nursery stock. New York (Cornell) Agr. Exp. Sta. Bul. 358. 1915.

portions and gives the fruit a decidedly dull appearance. Presumably as a result of excessive transpiration, the injured areas become somewhat depressed and the fruits definitely misshapen. The older lesions are reddish-brown and their skin texture is much tougher than that of healthy tissue.

In an attempt to analyze the environmental factors that had contributed to fruit infection, several diseased orchards were visited. They were located at an elevation 1200 to 1500 feet above those on the valley floor near Wenatchee, and the cherries generally mature 3 to 4 weeks later than those in the valley. During the evening of July 19, 1944, a heavy thunder shower occurred which was followed by hot, humid weather that lasted for approximately 1½ days. Prior to this shower, no fruit infection had been observed by any of the growers, but 2 days afterwards heavy infection was noticed in some of the orchards and in fruit being received at the packing house.

The trees in the orchard that was most severely affected were rather closely planted, and the tips of some heavily laden branches rested on the ground. It appeared that some of the trees might almost have functioned as giant moist chambers. Infection was so heavy on one of the trees that none of the cherries in the lower half were picked; with other trees lesser amounts of fruit were left because of the mildew. In the inner portions of the trees with heavy fruit infection there was invariably severe invasion of the succulent shoots and leaves. The presence of mature perithecia on some of the diseased leaves indicated that the initial attack had taken place rather early. Showers, interspersed with hot weather, during the middle of June and the first part of July probably promoted the initial leaf and shoot infection.

Another factor that possibly influenced the development of the disease in sweet cherries was the presence of mildew on wild choke-cherries (*Prunus demissa* (Nutt.) Walp.) growing in close proximity to the orchards. No significant morphologic differences could be found between the mildew on choke-cherries and that on sweet cherries. Tests to determine the pathogenicity of the choke-cherry mildew to sweet cherries have not been made. Growers report that frequently the choke-cherry is severely attacked by mildew when adjacent sweet cherries have little or no infection.

In the orchard that suffered greatest damage, the disease was far more severe on the Bing than on the Lambert or the Napoleon variety. In fact, several Lambert trees more or less surrounded by heavily infected Bing trees had only a trace of leaf infection and no mildew on the fruit. In an orchard at a slightly lower elevation in which picking largely had been completed, shoot infection was found on Bing, Black Tartarian, Lambert, and Napoleon varieties, and infected fruit was found on all varieties except Napoleon. Fruit inspectors reported that of the cherries examined by them in the packing houses, Black Tartarians appeared to be the most severely affected.

Local shippers inquired whether the mildew would continue to develop on the harvested fruit during storage and marketing, and whether the disease would predispose cherries to infection by decay fungi. To answer these

questions, 3 samples of cherries from a tree heavily infected with mildew were divided and stored at 40° F. and at 65° F., at a relative humidity of approximately 85 per cent. The samples were as follows: (1) All cherries infected with mildew, (2) no cherries visibly infected with mildew, (3) a composite of equal amounts of infected and visibly healthy fruits. A fourth sample similar to the latter was held in a moist chamber at 80° to 90° F. The cherries held at 65° or higher were examined after 5 days; those stored at 40° were examined after 10 days and again after 2 additional days at 65°. There was no evidence of an increase in size of the mildew lesions, nor were new infections found on any of the fruits that were visibly sound when stored. At 40° and at 65°, slightly more decay developed in mildewed than in sound fruit. However, since less than 4 per cent decay developed in the most severely affected sample, it appears that mildew does not markedly increase the susceptibility of the fruit to attack by decay fungi. Isolates obtained from rots centered at mildew lesions are arranged in decreasing order of prevalence, as follows: *Pullularia pullulans* (D By. and Loew) Berkh., *Botrytis cinerea* Fr., *Cladosporium sp.*, and *Rhizopus sp.*

Climatic conditions in the Wenatchee district during the summer of 1945 were entirely different from those in 1944, and the paucity of mildew in 1945 appeared to reflect these differences. During 1945 no precipitation was recorded at Wenatchee from June 9 to July 21, inclusive, and on July 22 only a trace of rain fell. Orchards that had had heavy mildew infection in 1944 were visited on July 6, 1945, but no sign of the disease was found on leaves, shoots, or fruits. Adjacent choke-cherries had slight mildew infection of shoots and leaves. A visit to the same orchards on July 27, as picking was being completed, revealed a trace of leaf, shoot, and fruit infection on Bing and Lambert varieties in one orchard. In another orchard, fruits on a sour cherry tree (Montmorency variety) had a trace of mildew, but no leaf or shoot infection could be found. The symptoms on sour cherry differed somewhat from those on sweet cherry in that the thin, almost indistinguishable mantle of fungus hyphae invariably radiated from the depression surrounding the stem.—HARLEY ENGLISH, formerly with the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Wenatchee, Washington. Present address: Botany Department, Oregon State College, Corvallis, Oregon.

*Hereditary Defects in the T.I. 448A Tobacco and Its Hybrids.*[1]—In breeding for resistance to one disease, there is always the hazard of increased susceptibility to another. During the development of flue-cured strains of tobacco resistant to bacterial wilt,[2] a parasitic disease and two hereditary defects have been observed on T.I. 448A and its hybrids. These abnormalities increased the difficulties of producing wilt resistant strains suitable for

[1] Cooperative investigations of the Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, the N. C. Agricultural Experiment Station and Department of Agriculture.

[2] Smith, T. E., E. E. Clayton, and E. G. Moss. Flue-cured tobacco resistant to bacterial (Granville) wilt. U. S. Dept. Agr. Circ. 727. 1945.

general culture, but it was possible by careful selection to isolate lines that were not affected by these troubles.

The occurrence of seedling blight associated with *Olpidium brassicae* (Wor.) Dang. (*Asterocystis radicis* de Wild.) was reported previously.[3] Two hybrid lines of T.I. 448A parentage were sown in a farmer's seed bed on unsterilized soil near Creedmoor, North Carolina. One strain sown on

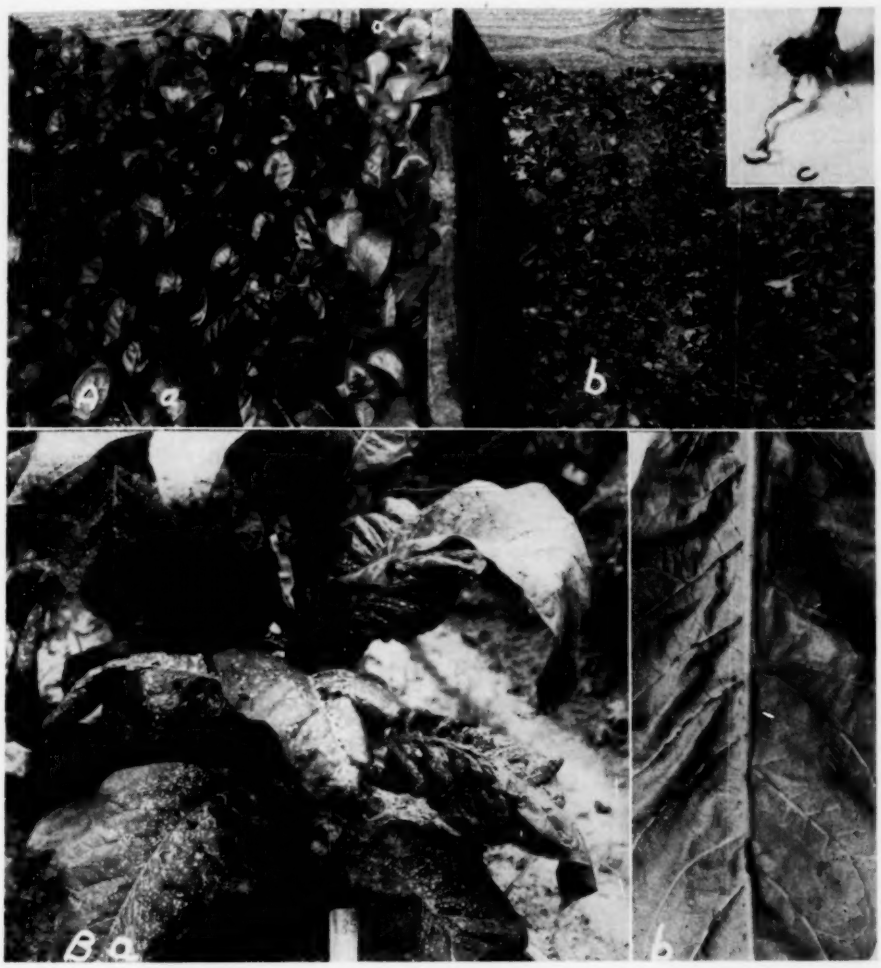


FIG. 1. A. Resistant strain (a) and T.I. 448A (b) grown on steam-sterilized seed bed; (c) root system of T.I. 448A. B. Leaf-spot-susceptible hybrid of T.I. 448A × 400 (a); vein necrosis (b).

20 square yards of bed grew slowly and over 90 per cent of the plants were killed in the 4 to 6 leaf stage by seedling blight. Major symptoms were yellowing and withering associated with brownish root decay. The disease was not observed on other wilt-resistant strains and standard varieties.

[3] Smith, T. E. Seedling blight on tobacco in North Carolina. U. S. Dept. Agr., Plant Dis. Reptr. 27: 273. 1943.

Another root disease occurred in seed beds at the Tobacco Branch Station, Oxford, North Carolina (Fig. 1, A). Major symptoms were stunted growth, brown root decay, and withering on hot days. This disease resembled seedling blight as well as ammonia toxicity. The cause is unknown but it was shown that neither *Olpidium* nor excessive rates of ammoniacal fertilizers was associated with it. All single-plant selections of T.I. 448A were susceptible. It was not possible to grow healthy seedlings on steam sterilized soil in beds outside or in the greenhouse. High soil temperatures reduced disease severity. Flue-cured varieties were resistant under all conditions. The $F_1$ of flue-cured varieties crossed with T.I. 448A grew normally, indicating that resistance was dominant. Susceptible $F_3$ and $F_4$ lines were found and eliminated.

An $F_2$ of T.I. 448A × flue-cured varieties showed 2.24 per cent of the plants with a leaf-spot disease in a population of 1518. White and brown necrotic lesions on the leaf blade combined with brown cankers of the primary and secondary veins were characteristic symptoms. Leaf breaking at the larger cankers on the primary veins also occurred. By selecting affected plants, uniformly susceptible lines were established. The cause is unknown. Washed tissue from young lesions was sterile on potato-dextrose agar and grafts failed to transmit the leaf spot. In crosses of susceptible and normal genotypes, the $F_1$ grew normally indicating that resistance was dominant. The $F_2$ from 3 such crosses averaged 10.86 per cent in a total population of 267 plants. The lesions on the secondary veins were similar to a "physiological weakness" reported as a recessive character by Johnson[4] in hybrids of Little Dutch and Cuban tobaccos.—T. E. SMITH, formerly stationed at the North Carolina Agricultural Experiment Station.

*Barley Fertilizer and Seed Treatment Test.*[1]—The 1941 barley crop of 49,954,000 bushels was the largest ever produced in Nebraska. By 1943 this production had dropped to 27,918,000 bushels. One of the major reasons for this drop was a severe attack of root rot, caused by unknown organisms. However, in view of the results obtained by several Canadian workers, preliminary fertilizer and seed-treatment tests seemed desirable in an effort to reduce the root rot damage. Greaney and Machacek[2] observed that the least amount of root rot of wheat caused by *Fusarium culmorum* (W. G. Swt.) Sacc. and *Helminthosporium sativum* P. K. and B. occurred in plots receiving a complete fertilizer. Russell and Sallans[3] found that phosphate fertilizers actually increased the amount of infection by the above mentioned organisms; however, the wheat receiving phosphate frequently outyielded

[4] Johnson, J. The inheritance of branching habit in tobacco. Genetics 4: 307–340. 1919.

[1] Published with the approval of the Director as Paper No. 418, Journal Series.

[2] Greaney, F. J., and J. E. Machacek. Progress report of the Dominion Botanist for the years 1931 to 1934, inclusive. Canada Dept. Agr., Div. Bot., 886 pp. 1935.

[3] Russell, R. C., and B. J. Sallans. The effect of phosphatic fertilizers on common root rot. Scient. Agr. 21: 44–51. 1940.

the non-fertilized plots in spite of the increased infection. Vanterpool[4] concludes that young wheat seedlings may be predisposed to fungal attack by the browning root-rot organisms at a critical stage in their development because of improper balance of available phosphorus and nitrate nitrogen in the soil. In later work,[5, 6] he reports that the addition of phosphate fertilizers reduced the damage from browning root rot and improved the growth of wheat seedlings.

Tests combining fertilizers and seed treatment were planted in 1945 in Merrick County in a fine sandy loam soil of low fertility and in 1946 in Hall and Buffalo Counties on sandy loam soils of moderate fertility. Table 1 indicates the nitrogen-phosphorus-potassium ratios used in 1945, and table 2 indicates the higher rates used in 1946. Nitrogen was supplied as Uramon, phosphorus as 45 per cent superphosphate.

TABLE 1.—*Barley fertilizer and seed treatment test planted April 6, 1945, in Merrick County, Nebraska*

| Treatment | Number of plants[a] | | Number of stems[a] June 6 | Yield[b] bushels per acre | Test weight (lb.) |
|---|---|---|---|---|---|
| | May 2 | May 17 | | | |
| None | 39.4 | 34.5 | 52.9 | 11.5 | 41.0 |
| 0–30–0 | 37.0 | 33.7 | 65.1 | 14.1 | 41.0 |
| 20–0–0 | 41.5 | 36.0 | 75.2 | 15.4 | 42.0 |
| 20–30–0 | 35.6 | 32.5 | 82.0 | 19.2 | 42.0 |
| New Improved Ceresan | 45.6 | 39.0 | 58.5 | 12.3 | 41.0 |
| 0–30–0 plus NIC | 40.6 | 33.7 | 56.7 | 12.2 | 39.0 |
| 20–0–0 plus NIC | 41.9 | 36.1 | 71.2 | 15.3 | 41.0 |
| 20–30–0 plus NIC | 38.0 | 33.6 | 90.7 | 24.0 | 43.0 |
| Av. of non-treated-seed plots | 38.4 | 34.2 | 68.8 | 15.1 | |
| Av. of treated-seed plots | 41.5 | 35.6 | 69.3 | 15.9 | |

[a] Average of two 5-foot lengths of row in each of four replications.
[b] Difference of 4.5 bushels required for significance.

Four replications of each fertilizer treatment were planted with certified Spartan barley, treated with New Improved Ceresan at the rate of ½ ounce per bushel, and four replications of each fertilizer treatment were planted with untreated seed. Plots were 4 feet wide and 100 feet long. The quadrat method of harvesting was used for yield determinations. Five units of one square yard each were taken from each replication.

In 1945, seed treatment produced no significant benefits until used in combination with the fertilizer treatment containing both nitrogen and phosphorus (Table 1). This fertilizer gave a significant increase in yield over the check when used without seed treatment. However, combined with seed treatment it gave even more significant results. The latter plot could be

[4] Vanterpool, T. C. Studies on browning root rot of cereals. III. Phosphorus-nitrogen relations of infested fields. IV. Effects of fertilizer amendments. V. Preliminary plant analysis. Can. Jour. Res. **13**: 220–250. 1935.

[5] ———. Studies on browning root rot of cereals. VI. Further contributions on the effects of various soil amendments on the incidence of the disease in wheat. Can. Jour. Res. (C.) **28**: 240–257. 1940.

[6] ———. Pythium root rot of grasses. Scient. Agr. 22: 674–687. 1942.

detected throughout the experiment by its greener color and more vigorous growth, whereas none of the other plots could be readily identified by observation.

Increase in yield from seed treatment was not obtained in the 1946 test (Table 2). Nitrogen produced significant yield increases in all plots, when used alone and in combination with phosphate, irrespective of seed treatment.

No significant stand differences were obtained in any of the tests as a result of seed treatment or the application of fertilizers. There were differences in the number of stems per 5-foot length of row as recorded in table 1. These differences appeared to be associated with the differences in yields obtained with the various treatments.

The failure to obtain benefits from seed treatment in the Buffalo and

TABLE 2.—*Barley fertilizer and seed treatment test planted April 4, 1946*

| Treatment | Number of plants[a] | | Yield[b] bushels per acre Buffalo Co. | Test weight (lb.) |
|---|---|---|---|---|
| | Hall Co. April 22 | Buffalo Co. April 22 | | |
| None | 56 | 66 | 26.0 | 46.0 |
| 0–40–0 | 54 | 62 | 29.2 | 46.5 |
| 40–0–0 | 57 | 59 | 34.7 | 44.5 |
| 40–40–0 | 51 | 55 | 42.1 | 45.5 |
| New Improved Ceresan | 53 | 62 | 27.4 | 47.5 |
| 0–40–0 plus NIC | 52 | 60 | 30.1 | 47.0 |
| 40–0–0 plus NIC | 47 | 54 | 37.2 | 45.5 |
| 40–40–0 plus NIC | 50 | 58 | 42.5 | 47.0 |
| Av. of non-treated-seed plots | 54.5 | 60.5 | 33.0 | |
| Av. of treated-seed plots | 50.5 | 58.5 | 34.3 | |

[a] Average of two 5-foot lengths of row in each of four replications.
[b] Difference of 4.2 bushels required for significance.

Hall County tests is not readily explainable. The soil was much higher in fertility than that represented in the 1945 test; and 1946 was also a much better year for barley production in Nebraska than was 1945. This may have been at least partially due to more favorable environmental conditions for the development of root rots in 1945. However, the results show that in certain years losses from root rot may be reduced by a combination of fertilizers and seed treatment. Although the damage from root rot fluctuates from year to year, the value of seed treatment from the standpoint of smut control alone makes seed treatment a practice to be highly recommended. When combined with fertilizer applications, it may become even more beneficial by reducing the damage from root rots.—J. E. LIVINGSTON, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

*Comparison of Benzene Vapor with Certain Sprays in the Control of Downy Mildew of Cauliflower.*[1]—In the United States, cauliflower (*Brassica*

[1] Contribution from Mississippi Truck Crops Branch Experiment Station, Crystal Springs, Mississippi. Published with the approval of the Director, Mississippi Agricultural Experiment Station. Paper No. 125, New Series.

*oleracea* var. *botrytis* L.) has been observed and reported[2, 3, 4] to be as susceptible as cabbage (*Brassica oleracea* var. *capitata* L.) to the downy mildew disease (*Peronospora parasitica* (Fr.) Tul.). Eddins[2, 3] has recommended Spergon (tetrachloro-para benzoquinone) and Fermate (ferric dimethyl dithiocarbamate) used as a spray or dust for the control of downy mildew on cabbage, under Florida conditions. Experimental data concerning control studies in relation to downy mildew on cabbage have shown that benzene when properly used will control downy mildew without apparent injury; certain sprays have also given satisfactory control. A preliminary report[5] dealing with benzene control of downy mildew on cabbage has been published.

The purpose of this paper is to show the contrast of the toxic effect of benzene vapor as compared with the beneficial effect of two organic sprays, Spergon (wettable) and Dow Seed Protectant No. 5 (tetrachloro-para benzoquinone) when used to control the downy mildew fungus on cauliflower.

In preliminary studies during the 1944–45 season benzene was used in a 66-square-yard bed to control downy mildew on cauliflower. Treatments at the rate of 50 cc. per sq. yd. on 5 successive nights per week under a wet cover of 48 × 44 thread count were started in advance of sporulation. Under severe mildew conditions this rate of treatment had effectively controlled downy mildew on cabbage. The results on cauliflower were unsatisfactory because benzene vapor failed to effectively control mildew sporulation and resulted in the death of many plants. The surviving cauliflower plants were stunted and developed slowly.

During the following seedbed season, 1945–46, benzene and the two organic sprays were compared in relation to effective control of downy mildew on cauliflower. Two cauliflower beds were sown on October 30, 1945; the same rate of seeding was used for both beds. One 10-yard bed was treated with benzene, 50 cc. per sq. yd. under a wet cover of 48 × 44 thread count. A total of 45 benzene treatments were applied, the first on November 8, 1945, and the final treatment on January 11, 1946. Treatments were started in advance of sporulation resulting from natural infection. The second bed (approximately 50 square yards) was divided into three sections; the center area remained the untreated control. The remaining two sections were sprayed bi-weekly with Spergon (wettable), 4 lb. per 100 gallons, and Dow Seed Protectant No. 5, 2 lb. per 100 gal., respectively. Orvus, at the rate of ½ lb. per 100 gal., was used as an emulsifier for both sprays. A total of 12 sprays was applied, the first on November 9, 1945, and the final spray on December 31, 1945. A few spray applications were necessarily omitted because of rain.

[2] Eddins, A. H. Control of downy mildew with Spergon and Fermate. Fla. Agr. Expt. Sta. Press Bull. 589. 1943.

[3] Eddins, A. H. Protecting cabbage plant beds from downy mildew with Spergon. Proc. Fla. Hort. Soc. 1944.

[4] Felton, Mathias W., and J. C. Walker. Environal factors affecting downy mildew of cabbage. Jour. Agr. Res. [U.S.] 72: 69–81. 1946.

[5] Foster, H. H., and J. A. Pinckard. Control of cabbage mildew by means of benzene vapor. (Abstr.) Phytopath. 34: 1000. 1944.

In these experiments Spergon (wettable) gave the best control, with a disease rating of 1 and average weight of 100 plant tops of 765 grams. Dow Seed Protectant No. 5 gave the second best control, with 1.5 for disease rating and 442 grams for weight of 100 plant tops. The benzene treatment was very unsatisfactory, resulting in the highest disease rating of 4 and causing severe plant injury resulting in an average weight of 99 grams for 100 plant tops. The untreated control plants gave a disease rating of 3 and a weight of 403 grams for 100 plant tops. Data are in table 1.

TABLE 1.—*Comparison of benzene vapor and certain sprays for the control of downy mildew on cauliflower*

| Treatment | Wt. of 100 plants (cut at soil line) | Average weight | Average disease rating[b] |
|---|---|---|---|
| | *Grams* | *Grams* | |
| Spergon (wettable),[a] | 811 | | |
| bi-weekly | 719 | 765 | 1 |
| Dow Seed Protectant No. 5,[a] | 410 | | |
| bi-weekly | 475 | 442 | 1.5 |
| Benzene, 50 cc. per sq. yd., | 103 | | |
| 5 successive nights per week | 96 | 99 | 4 |
| Untreated control | 450 | | |
| | 355 | 403 | 3 |

[a] Fungicides furnished without cost through the courtesy of United States Rubber Company, Naugatuck Chemical Division; and The Dow Chemical Company.

[b] Grading system for cauliflower plants of size for field transplanting.

0 = Vigorous growth, no sign of infection.

1 = Vigorous growth, no active fungus sporulation, 1 or 2 lower true leaves may occasionally show trace or slight necrosis. All true leaves holding well, stems bright.

2 = Rather vigorous growth with occasional lower true leaf showing necrotic lesions and with some chlorosis—occasional lower leaf may be ready to drop or have dropped, usually no active fungus sporulation, little or no kill of plants in early seedling stage, stems bright.

3 = Only fair to poor growth with more or less stunting observed, usually slight kill of plants during early seedling stage. Active fungus sporulation may or may not be present. Necrosis mostly general on true leaves, with one or more lower leaves usually dropped. Frequently more or less stem discoloration.

4 = Marked stunting, usually considerable kill of plants in early seedling stage. Active fungus sporulation may or may not be present. Most plants showing abundant necrosis on true leaves, lower leaves usually dropped. Plants frequently weak and with stem discoloration. Secondary rots may be present on stems and roots.

It is evident that benzene is unsatisfactory for the control of downy mildew of cauliflower. At the rate used and under the conditions of this experiment benzene was exceedingly toxic to cauliflower. The death of cauliflower plants appeared to have been due, in large part, to the toxic effect of benzene vapor. Cabbage plants, grown in an adjoining seedbed and treated with the same rate of benzene, remained vigorous and relatively free from mildew.

It is interesting that two Cruciferae (cauliflower and cabbage) react so differently to the benzene vapor treatment. Both cauliflower and cabbage can be protected from downy mildew by Spergon (wettable) and Dow Seed Protectant No. 5. There was some indication in this experiment that Spergon stimulated cauliflower plants to increased growth.—H. H. FOSTER, Mississippi Truck Crops Branch Experiment Station, Crystal Springs, Mississippi.

# REPORT OF THE 1947 ANNUAL MEETING OF THE SOUTHERN DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1947 Annual Meeting of the Southern Division of The American Phytopathological Society was held in part as a section at the meeting of the Association of Southern Agricultural Workers, January 15, 16, 17, in Biloxi, Mississippi. A joint session was held with the Crops Division. Over one hundred plant pathologists were in attendance and more than twenty formal papers were presented.

The Report of the Committee on Internal Cork of Sweet Potatoes was adopted with a recommendation that it be made a part of the Proceedings of the Biloxi meetings and printed with the minutes in Phytopathology. It was also adopted that a copy of the report be forwarded to the Director of the Agricultural Research Administration, U. S. Department of Agriculture. The Report follows:

A temporary committee composed of L. H. Person, T. T. Ayres, H. H. Foster, Coyt Wilson, James Epps, E. R. Stamper, C. E. Steinbauer, and C. J. Nusbaum met at the Hotel Biloxi on January 16 to discuss policy and program for the internal cork disease of sweet potato.

This committee made the following recommendations:

1. That, because of the regional scope of the problem, an official committee on internal cork of sweet potato be established by the Southern Division of The American Phytopathological Society.

2. That D. E. Ellis, J. H. Miller, and C. J. Nusbaum be proposed as members of this committee to serve until the next work conference is held.

3. That the committee take immediate steps to initiate surveys in the sweet-potato-growing states of the Southern region in order to determine the occurrence and severity of the disease, to propose uniform sampling techniques, to prepare uniform data cards for the inspecting agencies and to request the services of the U. S. Department of Agriculture in assembling, compiling, and disseminating the results of the surveys.

4. That each State make the best possible use of its existing facilities in conducting the survey and that inspectors be adequately instructed in the sampling technique and methods of taking data.

5. That the committee make a study of certification regulations as they apply to internal cork infection of sweet-potato seed stock and plants and that, meanwhile, each State take whatever steps deemed necessary in regard to restrictions designed to prevent the importation or intra-state movement of cork-infected planting stocks.

6. That plans for the initiation of collaborative research be deferred until further information on the occurrence of the disease is available.

7. That, if any state research agency contemplates undertaking a research project on internal cork of sweet potato the committee suggest that the project leader submit an outline of the project to the committee on internal cork in order that complete coordination of effort may be achieved.

C. J. NUSBAUM, *Chairman*

A business session was held on the morning of January 16 when the following officers were elected: *President*, A. L. SMITH; *Vice President*, C. J. NUSBAUM; *Secretary-Treasurer*, W. W. RAY; *Councilor*, S. G. LEHMAN.

Titles and abstracts of papers presented follow.

I. L. FORBES, *Secretary-Treasurer*

*Improving Wilt Resistance and Yield of Cotton by Roguing and Selection.* COTTON, JOHN R. Most wilt resistant strains of cotton have been developed from individual plant selections. The author has approached the problem from a different angle. Starting with a strain of low resistance and subjecting it to severe wilt conditions, by roguing and selection a highly resistant strain was developed having improved agronomic characters. The strain was a progeny from a D.&P.L.-Dixie Triumph cross. Bulk seed were planted in 1941 and roguing was done at 10-day intervals throughout the season. Only plants showing marked symptoms were removed, but the last roguing was made on the evidence of vascular discoloration. Some 300 plants were picked and studied in the laboratory for lint characters. From these, 100 plants were selected for planting in 1942. The same procedure was followed as in 1941. Only 76 selections were planted in 1943 and the same procedure was followed as in previous years. From the increase blocks in 1944, four

strains were selected for increase and testing. In a test at Baton Rouge in 1946 progeny No. 47–3–7–9 ranked first, having a lint percentage of 37.7, boll size of 85 bolls per pound, and lint length of 1 inch. Progeny No. 33–2–9–10 ranked second, having a lint percentage of 39.7, boll size of 80 bolls per pound, and lint length of $1\frac{1}{16}$ inch. Both strains had better than 98 per cent wilt resistance. This was in comparison to the original strain, which had a lint percentage of 35.9, boll size of 88 bolls per pound, and lint length of 1 inch and only 35 per cent resistance.

*A New Wilt-Resistant Watermelon.* EPPS, JAMES M. Fusarium wilt of watermelon causes losses in Tennessee ranging from a trace to as much as 100 per cent. Most varieties now grown are not resistant to wilt. In 1941 a breeding program was initiated and many crosses between resistant and nonresistant varieties have been made. Some have had high resistance. Some seed lots received from the Mississippi Station in 1942, most of which were open pollinated, when tested in greenhouse and field were highly resistant to wilt and possessed high quality. All lots were still segregating. Several of the most promising lots were planted in wilt infested soil and the resistant segregates self-pollinated. One lot, originating from a cross between Dixie Queen and Klondike and highly resistant in the greenhouse and field, has been self-pollinated and selections made for uniformity. In grower trials it has survived in wilt infested soil, where the previous year 100 per cent of a commercial variety died. This melon possesses high quality, has small white seed, is green with darker green stripes, is oblong, and has a tough rind. It is prolific and usually sets many fruits. Melons average about 25 lb., with some weighing as much as 40 lb. Grower trials have indicated that the melon is satisfactory from the standpoint of size, shape, quality, resistance, and color. It is being released as a variety and has been named Miles, honoring the late Dr. L. E. Miles, who initiated the work in Mississippi in 1935. The Tennessee and Mississippi Stations have cooperated in the watermelon work.

*Reaction of Species and Varieties of Cruciferae to Artificial Inoculation of Cabbage Downy Mildew.* FOSTER, H. H. In greenhouse and plant beds during the past three years approximately 100 commercial varieties representing several species of Cruciferae were tested for resistance to *Peronospora parasitica* in repeated experiments. Plants were artificially inoculated and kept under conditions favorable for sporulation. Infection was measured by a scale from 0 to 4. Under the conditions of these experiments all of the 60 commercial cabbage varieties tested were susceptible, falling in class 4 and occasionally class 3. All broccoli, Brussels sprouts, cauliflower, collard, and kohlrabi varieties tested were susceptible, class 4 or 3. All kale varieties tested, with one exception, fell in class 4. Siberian kale was resistant, falling in class 1, showing only slight necrosis. Rutabaga varieties varied from susceptible to tolerant. Four rutabaga varieties were tolerant, class 1 to 2, and one was susceptible, class 2 to 3. Turnip varieties tested varied from resistant to tolerant, in classes 0 to 2. Mustard varieties, with two exceptions, were highly resistant, classes 0 to 1; spinach and Chinese Broadleaf mustard varieties were less resistant. All radish and Chinese cabbage varieties tested were highly resistant, occurring in classes 0 to 1 with occasional slight necrosis on cotyledons but without sporulation. No variety tested was immune.

*Stem-end Rot Fungi Attack Immature Citrus Fruit.* HILDEBRAND, E. M. Stem-end rot fungi (*Phomopsis citri* and *Diplodia natalensis*) take a heavy toll of the Florida citrus (orange, grapefruit, tangerine) crop each year. Ordinarily infections develop only after mature fruit has been removed from the tree. As the result of this study, it appears that practically all developmental stages of citrus fruit from shortly after the blossom phase until maturity are naturally inoculated and become infected upon removal from the tree. The percentage of excised, immature fruits developing symptoms within one month from picking increased with the season from approximately 20 to 70 per cent between June and October. First symptoms developed on occasional fruits within a week. Others were still developing symptoms 16 weeks after removal. Histological studies of the button region of fruits of all ages frequently demonstrated the presence of stem-end rot mycelia or spores around, on, and under the calyx lobe. Since these mycelia were never detected below the cork layer on attached fruits, true penetration had not occurred. After fruits were picked, the fungus mycelia in the outer button tissues soon penetrated into the deeper button tissues and thence into the fruit. These studies support and extend those of Nadel (1944) on *Diplodia natalensis* in Palestine.

*Effect of Presoaking Unshelled Peanuts on Fungus Control, Germination, and Emergence.* IVANOFF, S. S. Soaking unshelled peanuts in water reduced fungus growth on the pods, decreased seed and seedling infection, and increased emergence in the field. In eight laboratory trials one lot of Spanish peanuts was soaked in water for 20 to 24 hours

and germinated in Petri dishes at 100 per cent humidity. A second lot (checks) of unsoaked peanuts was placed in Petri dishes at the time the first lot was put to soak. Records after 10 days showed 58 per cent of soaked pods with fungus growth and 96 per cent for the unsoaked pods. Species of *Fusarium*, *Penicillium*, and *Rhizopus*, *Sclerotium rolfsii*, and other fungi were present. In every trial the presoaked lot had fewer infected pods than the unsoaked lot. Average germination for the two treatments was 69 and 26 per cent, respectively. In four field trials emergence varied from no significant difference to great differences between treatments. In wet soil following heavy rains (2 trials) germination was about 35 per cent for both treatments. Shelled peanuts with and without Arasan treatment, showed no higher germination. In moist sandy soil, presoaked pods showed 77 per cent emergence for each of two trials, against 19 and 45 per cent for the unsoaked pods.

*A Virus-Induced Top Necrosis in Bean.* LEBEAU, F. J. A virus producing a severe systemic necrosis of bean, *Phaseolus vulgaris*, was isolated from bean pods from Crystal Springs, Mississippi, in September, 1944. Mechanical inoculation induced in bean seedlings a fine systemic chlorotic stippling which rapidly became necrotic, and was followed by early abscission of leaves and blossoms. Certain bean varieties were killed outright. In other varieties (Black Valentine, Bountiful, Tendergreen) the disease was not usually lethal and very young seedlings frequently recovered. Abundant proliferation of leaf buds of recovering plants produced a bunchy growth of mottled and malformed leaves and occasionally blossoms. Other susceptible legumes included *Phaseolus lunatus* var. Small White, *P. multiflora*, *Vicia sativa*, *Pisum sativum*, *Pisum arvense*, *Vigna sinensis*, *Soja max*, *Lupinus albus*, *Cyamopsis sporaloides*, *Melilotus indica*, *Crotalaria intermedia*, and *C. spectabilis*. *P. lunatus* var. Fordhook was susceptible only to local infection. In tobacco, local chlorotic ring spotting developed and occasionally systemic ring spotting. Infection in cucumber produced a fine chlorotic spotting and severe stunting. Infectivity of the virus was lost after infective sap was heated at 68° C. for 10 minutes. Striking similarities in the symptomology on several hosts and in the thermal inactivation points suggested that the bean virus was probably related to the soybean bud-blight virus and the guar virus reported by Chester and Cooper.

*Powdery Mildew of Soybean.* LEHMAN, S. G. Powdery mildew has on several occasions been reported on soybean in Europe and America. The causal fungus when named has been assigned to the genus *Erysiphe*. In 1936 and 1944, powdery mildew was found in the pathology greenhouse at Raleigh, N. Carolina. Perithecia present were of the genus *Microsphaera*. In 1944, powdery mildew was collected by Prince in Western North Carolina and reported as *Erysiphe polygoni*. When these specimens were given more careful examination, perithecia of the genus *Microsphaera* were found. In September, 1945, *Microsphaera* was found on soybeans at several locations in Eastern North Carolina. The disease was present in the same areas in 1946. The fungus produces short, club-shaped conidiophores. Perithecia are dark brown to black, sub-spherical, and have an average of about 20 appendages. Appendages are two to three times as long as the diameter of the perithecium, and are three to five times dichotomously branched. In field and greenhouse inoculations the following varieties were susceptible: Armredo, Cherokee, Herman, Ogden, Ralsoy, Seminole, Tokio, and a number of hybrid selections. Biloxi, CNS, Haberlandt, Roanoke, Rokusun, S-100, Volstate, and Woods Yellow, and several hybrid selections have remained free of mildew. (North Carolina Agricultural Experiment Station and Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Engineering, U.S.D.A., Cooperating.)

*Dithane on Cotton Root Rot in Field Trial.* MOORE, ELIZABETH J. Dithane (disodium-ethylene-bisdithiocarbamate) manufactured by Rohm and Haas was used in two forms, crystalline Dithane A-10 and a solution Dithane 14, in a field trial in an area heavily infested with the cotton root-rot organism, *Phymatotrichum omnivorum*. A Latin square of four treatments and a control replicated four times was designed. Dead plants were counted at regular intervals during the season. At the end of the summer, root systems were selected from each treatment for laboratory tests to determine whether the roots had absorbed any of this toxic material from the soil.

*A Possible Mosaic Disease of Cotton Observed in Louisiana in 1946.* NEAL, D. C. What may be a virus or mosaic disease of cotton was observed at the Louisiana Agricultural Experiment Station on August 30 in plants of the Mebane variety obtained originally from Texas in 1944 and subsequently grown in the greenhouse and field in 1945 and 1946. Noticeable symptoms are the following: loss of chlorophyll in whitish bands between the leaf veins, reduction in leaf size, lobed margins, and an unusual stiff appearance of affected plants. In general, the mosaic pattern is easily observed from a distance

in the field. On September 5, five additional Mebane plants in another field had varying degrees of the malady. Two plants were seriously affected—the upper and middle branches were malformed and sterile—but in each case normal branches with mature bolls were observed on one side near the base of the plants. This feature, that is, plants having both diseased and normal branches, was frequently encountered. Other plants also were discovered with only the terminal leaves involved, indicating that the infection may have been of recent origin. In later inspections, the disease was found in a nearby area on two other unrelated lines, namely, a Dixie-Triumph × Deltapine hybrid (36–25) and a selection of Stoneville 2–B. The fact that the disease occurs in separate lines would indicate that a virus may be the causative agent of this abnormality; and that it is not a deterioration due to genetic causes. Preliminary attempts to induce the disease by inoculating plants with sap extracted from leaves of affected plants have thus far been unsuccessful.

*Observations in 1945 and 1946 on the Relationship of Rainfall in Louisiana to the Incidence of Fusarium Wilt of Cotton.* NEAL, D. C. In 1945 and 1946 striking differences in rainfall and the prevalence of Fusarium wilt of cotton were noted at Baton Rouge, Louisiana. The rainfall in 1945—May through August—exceeded slightly the 20-year average, with higher departures occurring only in June and August. In 1946, it greatly exceeded this average, the departures being much higher for May and July and slightly higher for June. Maximum temperature ranges of 90° to 97° F. occurred each year during these months. In 1945 wilt appeared early, June 12, and became progressively worse as the season advanced; and crop failures resulted for several susceptible varieties. In 1946, even with the more susceptible varieties, only a scattering amount of wilt developed before the middle of August. The rainfall and bi-weekly wilt infection data indicate that the incidence of the disease is favored by alternately wet and dry periods during the optimum period for infection, June to August, and by rainfall slightly in excess of the 20-year average. In contrast, an extended period of excessive precipitation at this time is unfavorable for the disease.

*Studies of Boron Deficiency in Sweet Potatoes.* NUSBAUM, C. J. The influence of different rates of application of B at high and low levels of N, P, K, and Ca upon the incidence of B-deficiency, growth cracking, yield and quality of sweet potatoes was studied in a replicated field experiment. Variations of 3–9–9 fertilizer, which is the standard formula for sweet potatoes in South Carolina, included 0–9–9, 6–9–9, 3–9–0, 3–0–9, and 3–0–0, applied at the rate of 1,000 lb. per acre both with and without added lime. Borax was supplied at rates of 0, 5, and 20 lb. per acre. Without added borax, B-deficiency was severe with the low P (3–0–9), standard (3–9–9), and high N (6–9–9) fertilizers and was slight with the low N (0–9–9), low K (3–9–0), and low PK (3–0–0) fertilizers. Lime tended to increase B-deficiency. In all cases, B-deficiency symptoms were practically eliminated by an application of 5 lb. of borax per acre. Growth cracking was most severe with the low P (3–0–0), and high N (6–9–9) fertilizers and in most cases increased in proportion to the rate of applied borax. The highest yields and returns were obtained from the standard (3–9–9) fertilizer plus borax. With this fertilizer treatment, regardless of the Ca level, the returns from the 5 and 20 lb. applications of borax were about the same and generally exceeded the values for no borax by about $100.00 per acre.

*Studies of Internal Cork, a Probable Virus Disease of Sweet Potato.* NUSBAUM, C. J. Preliminary investigations of the nature and seasonal development of internal cork of sweet potato indicate that this disease is caused by a pathogenic virus. A constant association of a definite succession of foliage symptoms, indicative of a virus infection, with the internal cork symptom of the roots has been noted. Vein feathering and mottling of the leaves is followed by a reddish to purple splotching, sometimes in ring form, and then by a gradual fading out of leaf patterns. These leaf symptoms have been induced consistently in plants arising from healthy roots by making core grafts of corky root tissue into the healthy sweet potatoes. It has been shown that infected roots are able to carry the disease through storage and produce it in a succeeding crop grown from them. Although the disease appears to bring about premature deterioration of foliage, no noticeable reductions in yield have been noted. The corky areas in the flesh begin to appear about the time the first roots reach marketable size and these areas continue to increase in number and size in the field and to an even greater extent in storage. There has been considerable variation in the reaction of different varieties and strains of sweet potato, both as to the amount of corking of the roots and the character of leaf symptoms produced. Porto Rico and related strains have had considerable amounts of internal cork and Nancy Hall but little.

*Results of Seed Treatment in Controlling Damping-Off of Cotton in Mississippi.* PRESLEY, JOHN T. A study of the results of cotton seed treatments in Mississippi indi-

cates that no general statement will hold true for all conditions and soil types in the State. The unusually cool wet spring of 1946 was favorable for the development of damping-off and Thielaviopsis root rot of cotton seedlings. More rapid emergence and higher stand counts were generally obtained from acid delinted or reginned seed than from fuzzy seed, irrespective of treatment. All treated seed gave consistently better stands than nontreated seed. Dow 9 (zinc trichlorophenate) and Dubay 1452–F (7.7 per cent ethyl mercury p-toluene sulfonanilide) gave higher average stand counts than the remainder of the chemicals used. The loss from Thielaviopsis root rot this year was sufficient to warrant control measures, particularly for the Delta area of Mississippi. One of the more stable fungicides combined with the regular seed protectant may prove effective. (U. S. Department of Agriculture cooperating with Mississippi Agricultural Experiment Station.)

*Cotton Seed Treatment Tests in Oklahoma in 1946.* RAY, W. WINFIELD. Based on final seedling stands, no statistically significant difference between DuBay 1452–F (7.7 per cent ethyl mercury p-toluene sulfonanilide) and Dow 9–B (50 per cent zinc 2,4,5-trichlorophenate) was found when these two chemicals were applied to fuzzy-matted, reginned, reginned-matted, and acid-delinted seeds. All treatments were significantly superior to the nontreated fuzzy lot. The stands of seedlings from the greatest to the least were: acid-delinted, lightly reginned, heavily reginned, reginned-matted, fuzzy-matted, and fuzzy (nontreated). In the C–Test, involving 5 chemicals and 4 types of seeds (fuzzy, 2 degrees of reginned, and acid-delinted), DuBay 1452–F produced the best stands. Dow 9–5 (15 per cent Dow 9 and 85 per cent chloranil) and a dust composed of paraformaldehyde, benzoic acid, and dimethylolurea gave the lowest stands, whereas lots treated with a mixture of Fermate-DuBay 1452–F (6 parts to 1) and with 5 per cent mercury trichloroethylene were intermediate.

*Cotton Seed-Treatment Test.* ROGERS, C. H. Of 24 differently treated seed lots, emergence counts varied from a low of 52 per cent for nontreated fuzzy seed to a maximum of 91 per cent for acid-delinted seed or acid-delinted seed plus fungicidal dust treatment. No benefits were derived from matting seed regardless of further treatment. Seed that were reginned once were somewhat superior in performance to seed that were reginned four times. No significant differences were obtained in yields of seed cotton.

*Studies on the Seed Treatment of Rice.* RYKER, T. C., and S. J. P. CHILTON. Replicated rod row and field seed treatment tests on rice were conducted during the seasons 1943–1946 inclusive. The single rod-row tests, comprising three materials, Arasan (tetramethyl-thiuram-disulfide), Spergon (tetrachloro-parabenzoquinone), and New Improved Semesan, Jr. (organic mercury dust) and three dates of seeding, March, April, and May, were made at the Rice Experiment Station, Crowley, Louisiana. Stand counts and yield data were obtained. Five field tests with Arasan were planted with the drill that the grower was using on the farms. Stand counts were obtained by counting five rows ten feet long of treated and nontreated seed in four places. Arasan gave the best results, increasing stands nearly 25 per cent in the March and April plantings and 10 per cent in the May planting. Small but consistent increases in yield occurred where seed was treated. Arasan gave consistently better germination in seed stored for eight months and materially reduced the stored grain insect infestation.

*Cotton Seed-Treatment Studies in Georgia.* SMITH, A. L. Three-year trials of new dust disinfectants indicate DuBay 1452–F (6.5 per cent ethyl mercuri-p-toluene sulfonanilide) and Dow 9B (50 per cent of the zinc salt of 2,4,5-trichlorophenate) are satisfactory for cotton seed. DuBay 1452–F is equally as effective as New Improved Ceresan (5 per cent ethyl mercuric phosphate), now most commonly used, and is less vesicant and less disagreeable to workers. Dow 9B while slightly less effective than New Improved Ceresan is much less toxic to animals, less vesicant, and less disagreeable to workers. Fewer shank lesions occurred on seedlings treated with Dow 9B than with other materials, which suggests greater protection in the soil. Reginned seed produce 3 to 5 per cent fewer seedlings than fuzzy seed. However, ease of planting, more rapid emergence and better emergence with low soil moisture result in grower demand and widespread use of reginned seed. (Cooperative studies with the Cotton Seedling Disease Committee.)

*Wilt Resistance in Empire Cotton.* SMITH, A. L., and W. W. BALLARD. Empire is a promising new variety of cotton now in the third year of commercial production. The original parent plant was re-selected in 1935 from a field planting of Stoneville 2 originally selected in 1931. Selfing was started in 1938 and a number of lines have been selfed continuously since that date. Early field tests on wilt soil with pooled progenies indicated only moderate resistance. Tests of selfed lines in 1944, 1945, and 1946 showed a

wide range in resistance from high susceptibility to resistance comparable to that of the best commercial varieties. The wide range in resistance indicates the heterozygosity of the original plant and suggests its probable hybrid origin between Stoneville 2 and a wilt-resistant variety. The uniformity of the selfed lines for agronomic characters facilitated an immediate shift in production to those having high wilt resistance.

*The Effect of Soil Fumigation on Growth and Yield of Peach Trees.* TAYLOR, A. L., and C. W. MCBETH. Peach trees at Tifton, Georgia, planted in sites treated with chloropicrin to control root-knot nematodes (*Heterodera marioni*) had significantly larger trunk diameters after two years than those planted in untreated sites. Optimum size of site was apparently about 6 feet. Similar results were obtained where D–D was used instead of chloropicrin for nematode control. In another experiment peach trees in sites treated with chloropicrin and interplanted with cover crops susceptible to root knot (cowpeas and Austrian winter peas) produced 21.2 lb. of peaches per tree for their first crop, while trees in untreated sites with the same cover crops produced 4.8 lb. of peaches per tree. Trees in sites treated with chloropicrin and interplanted with cover crops resistant to root knot (*Crotalaria* and oats) averaged 41.9 lb. of peaches per tree, and trees in untreated sites with the same cover crop program produced 6.9 lb. of peaches.

*White Rot of Shallot and Its Control.* TIMS, E. C. White rot (*Sclerotium cepivorum* Berk.) occurs in the shallot-growing section of Southern Louisiana. The soil in a few fields is heavily infested with the fungus, which causes severe injury to shallots during the winter months. Small scale tests have been conducted over a period of three years to control the disease in the soil. A number of varieties and strains of shallot were tested for resistance. None of them showed any promise whatever. Lime was applied to infested soil in the field in sufficient amounts to change the pH from 5.6–5.8 to about 7. The amount and severity of infection was reduced to some extent, but satisfactory control was not obtained. Certain other chemicals applied in small amounts to the soil around the shallot plants gave promising results. Semesan (30 per cent hydroxymercurichlorophenol) at the rate of 1 oz. per gallon of water and mercuric chloride (1–500) applied at the rate of 80 cc. per plant gave almost complete control. A number of the common seed treatment compounds were used for dusting shallot seed sets before they were planted in diseased soil. None of them reduced the amount of white rot to any appreciable extent.

*Symptoms Induced at Standardized Wounds by Fungi Isolated from Dry Turpentine Faces.* TRUE, R. P., and MILTON M. SMUCKER. *Diplodia pinea* (Desm.) Kickx, *Glocotulasnella pinicola* (Bres.) Rogers, and *Ceratostomella ips* Rumbold produced pitch soak and dry face (cessation of normal gum flow after wounding) at cambium-depth inoculated wounds in slash pine (*Pinus caribaea* Morelet). *Diplodia natalensis* Evans induced considerable pitch soak but little dry face. No notable symptoms resulted from inoculating wounds with species of *Cytospora*, *Trichoderma*, *Penicillium*, *Geotrichum*, *Schizophyllum*, *Stereum*, and seven undetermined fungi. The three dry-face fungi and possibly *Diplodia natalensis* are considered capable of invading uninfected turpentine faces. The other fungi tested are regarded as secondary invaders usually incapable of infecting healthy faces. Gum yield and pitch soak measurements showed that temporary gum flow stimulation and extensive pitch soak symptoms usually preceded the dry face condition. Symptoms were most pronounced following inoculations with *Diplodia pinea*. Wound response differences were largely attributable to the different fungi tested, but individual tree characteristics also determined in part the type and severity of symptoms. Present experimental evidence indicates that certain fungi may often have a primary rôle in inducing dry face, a major source of loss to the naval stores industry.

*Relation of Environment to the Incidence of Fusarium Wilt of Cotton.* YOUNG, V. H. One hundred ninety-two soil samples from eleven areas in Arkansas failed to show correlation between the pH of the soil and cotton wilt. The pH varied from 4.66 to 8.4. Wilt was severe on potash-deficient areas which are often acid, but acidity did not appear to be the governing factor. In certain areas cotton responded to applications of boron but the incidence of cotton wilt was not lowered by the use of fertilizers containing boron. Records at the Cotton Branch Experiment Station at Marianna, Arkansas, taken for eighteen years showed that high rainfall, especially prior to August, was generally correlated with higher incidence of Fusarium wilt of cotton whereas in drouth years or years with prolonged dry periods during June and July there was less wilt. In the 5-year period from 1929 to 1933 cotton was planted at 2-week intervals from April 15 to July 1. The 5-year average for April 15 plantings was 35 per cent wilted plants in contrast to 3 per cent for the July 1 plantings. High soil moisture during more of the growing season is believed to be the principal cause of higher wilt incidence in early planted cotton.

# REPORT OF THE FOURTH ANNUAL MEETING OF THE POTOMAC DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meeting was held on February 19 and 20, 1947, at the Plant Industry Station, Beltsville, Maryland. Some 75 members were in attendance. In addition to the 29 research papers presented, the program included the following: A survey of the results being obtained with the newer fungicides, H. P. Barss and committee; plans for more effective disease reporting, P. R. Miller and R. J. Haskell; the golden nematode situation in this country and in England, W. S. Popham. Demonstrations were given of the slurry method of seed treatment, G. F. Miles, and of aerosol fumigation for greenhouses, F. F. Smith. A dinner, attended by 85 members and guests, was followed by an evening program of motion pictures provided through the courtesy of the Sherwin-Williams and the E. I. Du Pont Companies, and an illustrated lecture on Plant Virus Research in England and Ireland by Dr. R. H. Larson of the University of Wisconsin. Officers elected for 1947–1948 were V. F. TAPKE, *President;* W. F. JEFFERS, *Vice President;* W. W. DIEHL, *Secretary-Treasurer;* and E. E. CLAYTON, *Councilor.*

## ABSTRACTS OF PAPERS

*Black Rot as a Storage and Market Disease of Sweet Potatoes.* COOLEY, J. S. The recent expansion of the marketing of freshly-dug, non-cured sweet potatoes and the practice of washing in preparation for marketing give new emphasis to the importance of investigations now in progress on the black-rot disease in the market. When black rot is present in the crop at digging time it will continue to develop during storage. It will even develop in non-cured roots during the relatively short time they are being marketed. Washing of non-cured sweet potatoes, with the unavoidable skinning, favors infection and spread of the disease. It is important that growers know how to handle this crop when black rot is present in it at digging time in such a way that the black-rot losses will be minimum. Considering the possibility of continued development of black rot, less loss will occur when the sweet potatoes are sold in an active nearby market as soon as dug. When such a market is not available it is preferable to cure for 10 days immediately after digging and grade out the rots that developed during curing; then market as soon thereafter as possible. A campaign for black-rot control by all States producing sweet potatoes should greatly reduce present heavy loss from this disease.

*A Proliferating Gall on Blueberry Plants Caused by an Actinomyces.* DEMAREE, J. B. A fungus tentatively identified as an *Actinomyces* causes a tumefaction often accompanied by bud proliferation at or near the primary node of blueberry (*Vaccinium*) plants. The aberrant growth often attains a huge size in comparison with that of the infected stem. The numerous buds emerging from the gall tissues usually become aborted early, or a few buds may differentiate into short fleshy shoots with abnormal leaves, or sometimes several buds develop into spindly shoots with small but normal-sized leaves, giving rise to a growth resembling a witches'-broom. The fungus readily infected young blueberry plants when bits of agar containing hyphae and spores were applied to injured tissues in the region of the primary node. It was reisolated from artificially infected plants and in turn was used successfully as inoculum. The fungus was originally isolated from naturally induced galls on young blueberry plants grown in soil composed partly of leaf mold taken from a forest floor where the huckleberry (*Gaylussacia baccata*) grew. Among several different lots of seedling blueberry plants of different parentage, totaling over 1,700, the incidence of natural infection per lot varied from 8 per cent to 70 per cent, an average of 26 per cent. Many of the huckleberry plants in the woodlot where the propagating soil was obtained have an undetermined witches'-broom disease.

*Germination of Oospores of Pythium Butleri and Pythium tardicrescens.* DRECHSLER, CHARLES. When transferred to a shallow layer of distilled water, oospores of *Pythium Butleri* from maizemeal-agar plate cultures 60 days old were found to germinate freely by the production of zoospores. Preliminary to such germination the massive oospore wall is commonly reduced to a thin outer layer through resorption of a thicker inner portion, while the protoplasm loses the organization characteristic of the resting condition to present an appearance much like that in the spherical sporangium of *P. debaryanum.* The granular contents, on being discharged into a vesicle at the tip of a simple or occasionally somewhat branched evacuation tube commonly 40 to 225 μ long and 2.5 to 5 μ wide, are transformed into approximately 10 motile zoospores. Oospores from a 112-day-old culture of *P. tardicrescens* that was isolated from a blackened root of *Bidens sp.* gath-

ered in Arlington, Va., Oct. 11, 1943, likewise germinated by producing about 10 zoospores in a vesicle at the slightly reflexed orifice of a simple or occasionally somewhat branched evacuation tube, which here commonly measured 60 to 350 μ in length and 2 to 3 μ in width. After discharge the persisting outer layer of the oospore wall in each species sometimes encloses a separate sporangial envelope.

*Some Effects of Spergon as a Pea Seed Treatment on Soil Fungi.* HASSAN, H. H., and C. E. COX. The value of Spergon (tetrachloro-para-benzoquinone) seed treatment in improving stand, reducing damping-off and root rot of pea seedlings is known. Its effect on soil-inhabiting fungi apparently has not been investigated. The number of fungi which could be cultured from a sample of soil planted with Spergon-treated pea seed was less than half that from a similar sample of soil planted with untreated seed. When aliquots of soil were exposed to various concentrations of Spergon in soil suspension for varying periods of time before culturing, it was found that a 1–100 concentration inhibited all growth after momentary exposure. A 1–1000 concentration permitted some colonies to develop up to 24 hours, but with longer exposures prevented all growth. Weaker doses of Spergon prevented the growth of increasingly greater numbers of fungi with increasing exposure time. The pea pathogens, *Pythium sp.*, *Rhizoctonia solani*, *Ascochyta pinodella*, and *Fusarium solani* f. *pisi* were cultured on Czapeck's agar medium containing various concentrations of Spergon. Concentrations of 1000 p.p.m. and 100 p.p.m. Spergon in the medium killed *Pythium sp.*, and reduced the growth rate of *A. pinodella* and *F. solani* f. *pisi*. Growth of *R. solani* was prevented on medium containing 1000 p.p.m. Spergon, but the fungus was not killed.

*"Specificity" of the Metallic Dithiocarbamates in the Control of Certain Vegetable Diseases.* HEUBERGER, J. W. Research conducted in Delaware (1943–1946, incl.) on the development of metallic dithiocarbamates as fungicides has concerned itself with a study of various metallic salts in the dimethyl series, a comparison of similar metallic salts in the dimethyl and ethylene series, and an evaluation of these materials for control of several vegetable diseases. Data obtained show the following: (1) in the dimethyl series, the zinc salt (Zerlate) was more effective than the sodium, calcium, iron (Fermate), copper, and lead salts for the control of early blight on tomato and potato, was more effective than the sodium, calcium, and iron salts for control of tomato anthracnose, and was more effective than the iron salt for control of downy mildew on cucumbers; (2) in the ethylene series, the zinc salt was more effective than the iron salt for control of early blight and late blight on potato and tomato, for tomato anthracnose, and for downy mildew on cucumbers; (3) the zinc and iron salts in the ethylene series were more effective than corresponding salts in the dimethyl series for control of early blight and late blight on potato and tomato and for downy mildew on cucumbers, but were slightly less effective against tomato anthracnose.

*Evaluation of Several Fungicides in Preventing Infection of Sweet Potato Slices by Ceratostomella fimbriata.* JEFFERS, W. F. On the basis of percentage active ingredient, a dilution series, of 100 g. per liter to 0.001 g. per liter, was prepared for each of several fungicides. Slices of Maryland Golden sweet potato were placed in an inoculum of the black-rot organism and then dipped in the fungicide solutions. These pieces were then placed in moist chambers and incubated at 28° C. for four days. By this time the surface of slices dipped in water was covered with growth of *Ceratostomella fimbriata* and there was heavy production of ascospores and conidia. Puratized N5-E (phenyl mercury triethanol ammonium lactate), Isothan Q-15 (lauryl isoquinolinium bromide), mercuric chloride, Semesan Bel (hydroxymercurinitrophenol) and Puratized 177 (para amino phenyl cadmium dilactate) showed high toxicity to the organism. Borax, wettable Spergon (tetrachloro-para-benzoquinone), Zerlate (zinc dimethyldithiocarbamate), and Phygon (2,3 dichloro 1,4 napthoquinone) were moderately effective. Copper 8 hydroxyquinolate, Fermate (ferric dimethyldithiocarbamate) and zinc ethylene bis dithiocarbamate were only slightly effective. Manganese ethylene bis dithiocarbamate and Tersan (tetramethylthiuramdisulfide) were of little value in preventing infection. In general the materials giving best control of the black-rot organism caused most injury to sweet potato tissue.

*Diseases with Scab-like Symptoms Observed in Southern California in 1946.* JEHLE, R. A., and ANNA E. JENKINS. The senior writer examined ornamentals in Southern California for diseases with scab-like lesions during November and December, 1946. No violet scab (*Sphaceloma violae*) was found. Diseases with similar symptoms were prevalent and destructive on other ornamentals, namely *Hedera helix* (English ivy), growing as a lawn cover in Los Angeles, Pasadena, Altadena, Fullerton, and Redlands; *Pelargonium peltatum* (ivyvine geranium), lawn plantings in the same localities, also in Santa Ana, and *Schinus molle* (California pepper tree), planted on streets in Los Angeles. *H.*

*helix* and *S. molle* were severely defoliated. Representative specimens were referred to the junior writer who reported: The disease of *H. helix* is called "scab of English ivy" (*Sphaceloma hederae* Bitancourt and Jenkins (1946)). That of *P. peltatum* requires further study for exact diagnosis. Its symptoms resemble Ringuelet's (1927) intumescences on *P. peltatum*, also those of the so-called "dropsy" or "oedema" of common *Pelargonium*, in one instance, at least, reported (F. C. Stewart, 1910) on *P. peltatum*. What is probably *Macrosporium pelargonii* Ell. & Ev., originally described on *Pelargonium sp.* from Pasadena, is present, although apparently nonpathogenic. Leaf lesions on *S. molle* are consistent with a *Sphaceloma* disease. In January, H. S. Fawcett sent specimens of all three diseases from other localities in Southern California.

*Additional Records on Distribution of Plantain Scab.* JEHLE, R. A., E. A. WALKER, D. A. PRESTON, and ANNA E. JENKINS. Jenkins and Bitancourt in 1946 described a new species, *Sphaceloma plantaginis*, causing plantain scab. This was based on herbarium material (oldest specimen collected at Still Pond, Md. 1891), and recent collections since 1939, mostly on *Plantago spp.* and *P. rugelii*, representing 62 collections from 14 States east of the Mississippi River and from the District of Columbia. A survey in Maryland during 1946 extends the known distribution of scab on *P. rugelii* to 36 localities (in 16 counties) of which 30 are new. One herbarium specimen was found showing scab dating back to 1905. Specimens collected from two States west of the Mississippi River, Minnesota and Oklahoma, extend the known distribution to sixteen States and the District of Columbia. Additional collections were reported from Virginia and West Virginia to make collections now available from 102 locations. In Maryland, plantain scab was very abundant on *P. rugelii*, being most severe on plants located along sidewalks, roadways, and parking lots. In open fields scab was less severe and more difficult to find. Two specimens of *P. lancelata* having scab-like lesions suggesting plantain scab were collected in Virginia and Maryland.

*Periconia circinata (Mangin) Sacc. as a Possible Causal Factor in "Milo Disease."* LEUKEL, R. W., and FLORA G. POLLACK. Isolations from the roots of Colby milo grown in soil from fields naturally infested with "milo disease," frequently yielded a fungus with a dark mycelial growth producing either abundant conidiospores or chains of thick-walled chlamydospores. The predisposing factors causing these variations in sporulation have not been extensively studied, but they seem to include the type and depth of the media used, the presence of contaminants, and other conditions of environment. This distinctive fungus was identified as *Periconia circinata* (Mang.) Sacc. Heavy inoculation with this fungus in steamed soil caused severe root injury in Dwarf Yellow and Colby milos, susceptible to the "milo disease," but had no apparent effect on milos resistant to "milo disease." In soil less heavily inoculated, susceptible milo plants matured, but were stunted, produced small heads or none at all, and died considerably in advance of milo plants resistant to "milo disease," and grown in the same soil. The susceptible plants resembled in many respects those grown in soil naturally infested with the "milo disease" and this fungus may be a contributing causal agent of that disease.

*Effect of Temperature and Moisture on the Efficacy of Soil Fumigants.* MCCLELLAN, W. D., J. R. CHRISTIE, and NORMAN L. HORN. The effect of soil temperature and moisture on the efficacy of Larvacide (chloropicrin), D-D (dichloropropene and dichloropropane), Dowfume G (methyl bromide), and Dowfume W-15 (ethylene dibromide) was investigated by means of Wisconsin-type soil temperature tanks. Results were based on the effect of 2.5 ml. of each fumigant on three test organisms placed in the soil seven inches from the point of injection. The effect of Larvacide was most rapid at 98° F. It was only slightly effective against *Heterodera marioni* at lower temperatures. D-D was not effective against the fungi, but gave complete nematode kill after one day's exposure at 82° or 98° F., and after three days at 62° or 72°, but very little at 45° or 55° even after 13 days. Dowfume G was not effective against *Fusarium oxysporum* f. *callistephi*, only slightly effective against *H. marioni*, but had considerable effect against *Sclerotium rolfsii* at 72°, 82°, or 98°F. Dowfume W-15 was not effective against *F. oxysporum* f. *callistephi* but was effective against *S. rolfsii* at 72° F., and above after six days. Against *H. marioni* it was most effective at 98° F., and was effective at all temperatures after three days. In general, all four fumigants were most effective, and were retained in the soil for longer periods, when the soil was wet.

*Survival of Labile Viruses in Desiccated Leaf Tissue.* MCKINNEY, H. H. Seven species of labile viruses have been desiccated over $CaCl_2$ at 1.5° C., and then stored in tight bottles without desiccant at 1.5° C. Atmospheric oxygen was not removed. All species showed activity for periods of 177 days and longer. Viruses of tobacco ring-spot, Southern celery mosaic, and cucumber mosaic were highly active after 547, 765, and 745 days, respectively. Viruses of potato "Y" mosaic and prairie wheat yellow mosaic were

moderately active after 420 and 701 days, respectively. When stored at 26° C., activity was lost or very low after two weeks. Tests with the tobacco ring-spot virus show slight survival after 13 weeks storage at 26° C., when desiccation was completed over $Mg(ClO_4)_2$ (anhydrone) at 1.5° C., and continued with the same desiccant during the storage period. It appears that the reactions favoring rapid inactivation of virus when stored at laboratory temperatures, are favored by extremely small quantities of water in the tissues. Complete desiccation should facilitate survival of these viruses during transportation, and during storage in the event of breakdown in a refrigeration system. Since the method greatly reduces the stocking of viruses in growing plants, and the chances of contamination, it should serve as a distinct aid in extending the type-culture method to labile plant viruses.

*Wheats Resistant to and Immune from Mosaic in Nursery Tests Are Susceptible When Inoculated Manually.* MCKINNEY, H. H. Nine lines of winter wheat that have shown either very high resistance or immunity against *Marmor tritici typicum* and *M. t. fulvum* in field tests have been grown in noninfested soil in the greenhouse at temperatures favoring infection. When these lines were inoculated with fresh extracts of the viruses mentioned, strong mosaic symptoms developed in each. The percentage of infected seedlings was sometimes less and sometimes greater than in Michigan Amber, the field-susceptible control variety. Seedlings failing to show mosaic have, on reinoculation, developed mosaic, showing that they escaped infection from the previous inoculation. The results suggest that these wheats possess quantitative resistance to virus, or that they are resistant or immune against some viriliferous vector which inhabits the soil. On the contrary, similar tests with four lines of winter barley that are immune from mosaic in the field indicate that a high percentage of the plants is either highly resistant or completely immune against virus applied by manual inoculation.

*Some Little-Known Nematodes Parasitic on Roots.* STEINER, G. A cyst-forming *Heterodera* species, which might be mistaken for *H. rostochiensis*, is widely distributed in the potato fields of our eastern and central States. It appears to attack exclusively various polygonums. Normally of lemon shape with a well-marked vulva, certain form variations of the cyst of this knotweed *Heterodera* closely resemble cysts of the golden nematode. The cyst wall, however, shows a linear meandrous ornamentation quite different from that of *H. rostochiensis* with its rows of dots. There is evidence of significant root damage by various species of *Hoplolaimus*. These are large forms causing extensive mechanical injury. Such damage is demonstrated on *Pinus taeda* seedlings. On the roots of a declining gardenia a minimum number of 335 such nematodes was counted per inch. Nematodes that attack roots from the surface have not been given the consideration they deserve. Spiral nematodes (*Helicotylenchus* and *Rotylenchus*), by puncturing the roots, appear regularly to initiate extended necrosis. These forms, dropping from the roots if disturbed, have been largely overlooked. *Rotylenchulus* is a later evolutionary stage of a mode of life initiated by these spiral nematodes: here a permanent fixation on a root surface is attained. Motility is lost and obesity follows sedentary life. Cotton, tobacco, coffee weed, and yew are examples of hosts in the United States.

*Combination of Cercospora Leaf-Spot Resistance and Curly-Top Resistance in Sugar-Beet Varieties.* STEWART, DEWEY. The leaf-spot-resistant sugar-beet variety U.S. 216, extremely susceptible to curly-top, was crossed with S.L. 611, U.S. 12, and U.S. 22/1, mass selected curly-top-resistant varieties that are extremely susceptible to leaf spot. The $F_1$'s were then crossed with U.S. 22/1, thereby essentially producing a back cross in which the curly-top-resistant variety was the recurring parent. Selections were made from the back-cross generation under conditions of severe leaf-spot exposure, and a further selection for leaf-spot resistance was made in the progeny of this selection. Seed from the second selection for leaf-spot resistance was directly increased at State College, New Mexico, by the field overwintering method. S.P. 3–6–0 tested in 1944 showed curly-top-resistance essentially equal to U.S. 22/1 and only slightly less leaf-spot resistance than U.S. 216. S.P. 456–0, produced in the same manner, when tested in 1946 showed curly-top and leaf-spot resistance essentially equal to that of the respective resistant parents.

*Influence of Different Levels and Combinations of Nitrogen, Phosphorus, and Potassium on the Susceptibility of the Tomato Plant to Infection by Alternaria solani.* THOMAS, H. REX. Varying the levels and combinations of nitrogen, phosphorus, and potassium in sand-culture greenhouse tests affected the response of tomato plants to infection by *Alternaria solani*. Plants grown on the high level of phosphorus had less defoliation and smaller leaf spots than the plants on the low-phosphorus level. Although plants grown on the high level of nitrogen had larger leaf spots than plants on the low-nitrogen level, there was no significant difference in defoliation between the two levels. Plants grown on the high-potassium level had a greater percentage of dead leaves and larger leaf spots

than plants grown on the low levels in two of the three experiments. A significant interaction between nitrogen and phosphorus was found in the amount of defoliation. With high nitrogen and low phosphorus the plants had the most defoliation and with high nitrogen and high phosphorus, the least. Plants grown in solutions medium to high in nitrogen, low in phosphorus, and medium to high in potassium were usually the most susceptible to disease development. The leaf spots were larger on the older leaves than on younger leaves, regardless of the plants' apparent nutritional condition.

*Comparative Action of Viruses and Mutant Chondriogenes on Plant Cells.* WOODS, M. W., A. F. WOODS, and H. G. DUBUY. Mutant chondriogenes affect cells in three distinct ways, (1) qualitatively change the structure and function of plastids possessing them, (2) quantitatively change development and function of the non-mutated plastids of the same cell, and (3) alter function and development of the cell as a whole. At least 5 different types of mutant plastids have been observed in a single species. Infection of *Nicotiana tabacum* L. with strains of tobacco-mosaic virus results ultimately in cytological changes paralleling those induced by mutant chondriogenes. In mature cells the virus first affects all of the plastids, reducing their size and pigment content. Physiological disturbances also occur. In time, however, a variable number of plastids become more or less normal in appearance the remainder undergoing profound metamorphosis. Such metamorphosed plastids closely resemble those with mutant chondriogenes. Plastid metamorphosis occurs most rapidly in young systemically infected leaves. In all cases cells occur in which some of the plastids do not undergo such metamorphic change. In chloroses induced by nitrogen starvation or colchicine all of the plastids in a given cell showed general degeneration, and none of the specific changes described for viroses and variegations. Whereas tobacco-mosaic virus is infectious, mutant chondriogenes have not displayed this property.

Following are titles of other research papers presented at the fourth annual meeting of the Potomac Division, the abstracts of which will be published elsewhere:

*Preliminary Tests to Determine the Nematocidal and Fungicidal Properties of Certain Chemical Compounds When Used as Soil Fumigants.* J. R. CHRISTIE.

*Recent Advances in the Breeding of Disease-Resistant Tobaccos.* E. E. CLAYTON.

*Phytotoxic Effect of DDT and Other Chlorinated Hydrocarbons.* ARTHUR C. FOSTER.

*Structures Corresponding to Appressoria and Substomatal Vesicles Produced on Nutrient-Solution Agar by Germinating Urediospores of Cereal Rusts.* ANNIE MAY KARRER and H. A. RODENHISER.

*Inheritance of Resistance to Bacterial Canker in Cow Peas.* C. L. LEFEBVRE and HELEN S. SHERWIN.

*Dusting of Fruit Trees with Special Reference to Methods of Application.* A. A. NIKITIN.

*Evidence of Fusion Bodies from Urediospore Germ Tubes of Cereal Rusts.* H. A. RODENHISER and ANNIE MAY KARRER.

*Physiologic Specialization of Phytophthora Infestans.* E. S. SCHULTZ and LILLIAN CASH.

*Recent Advances in the Breeding of Disease-Resistant Potatoes.* F. J. STEVENSON.

*Myrothecium as a Tomato Fruit Rot Organism.* J. A. STEVENSON and L. P. MCCOLLOCH.

*New Methods of Artificial Seed Inoculation for Testing the Resistance of Spring and Winter Barleys Against Stripe, Helminthosporium gramineum.* V. F. TAPKE.

*A New Local Lesion-Producing Virus of Beans.* W. J. ZAUMEYER and H. REX THOMAS.

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